A STUDY OF CLINICOPATHOLOGICAL FEATURES AND PROLIFERATION MARKER KI-67 EXPRESSION IN OCULAR SURFACE SQUAMOUS NEOPLASIA

Dissertation submitted in

partial fulfilment of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

INSTITUTE OF PATHOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

OCTOBER 2017

CERTIFICATE

This is to certify that this Dissertation entitled **"A STUDY OF CLINICOPATHOLOGICAL FEATURES AND PROLIFERATION MARKER KI-67 EXPRESSION IN OCULAR SURFACE SQUAMOUS NEOPLASIA"** is the bonafide original work of **Dr.GOKULAKANNAN.R**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in October 2017.

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DECLARATION

I, **Dr.GOKULAKANNAN.R,** solemnly declare that the dissertation titled "**A STUDY OF CLINICOPATHOLOGICAL FEATURES AND PROLIFERATION MARKER KI-67 EXPRESSION IN OCULAR SURFACE SQUAMOUS NEOPLASIA"** is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **Prof.Dr.RAJAVELU INDIRA, M.D.,** Professor of Pathology,Institute of Social Obstetrics and Govt Kasturba Gandhi hospital, Madras Medical College.. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr.GOKULAKANNAN.R**

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

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CERTIFICATE OF APPROVAL

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To
Dr.Gokulakannan.R.
III Year PG in MD Pathology
Institute of Pathology
Madras Medical College
Chennai 600 003

Dear Dr.Gokulakannan.R,

The Institutional Ethics Committee has considered your request and approved your study titled "**A STUDY OF CLINICOPATHOLOGICAL FEATURES AND PROLIFERATION MARKER Ki-67 EXPRESSION OCULAR SURFACE SQUAMOUS NEOPLASIA " NO.30032017(I)**

The following members of Ethics Committee were present in the meeting hold on **02.03.2017** conducted at Madras Medical College, Chennai 3

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9.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
10.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee
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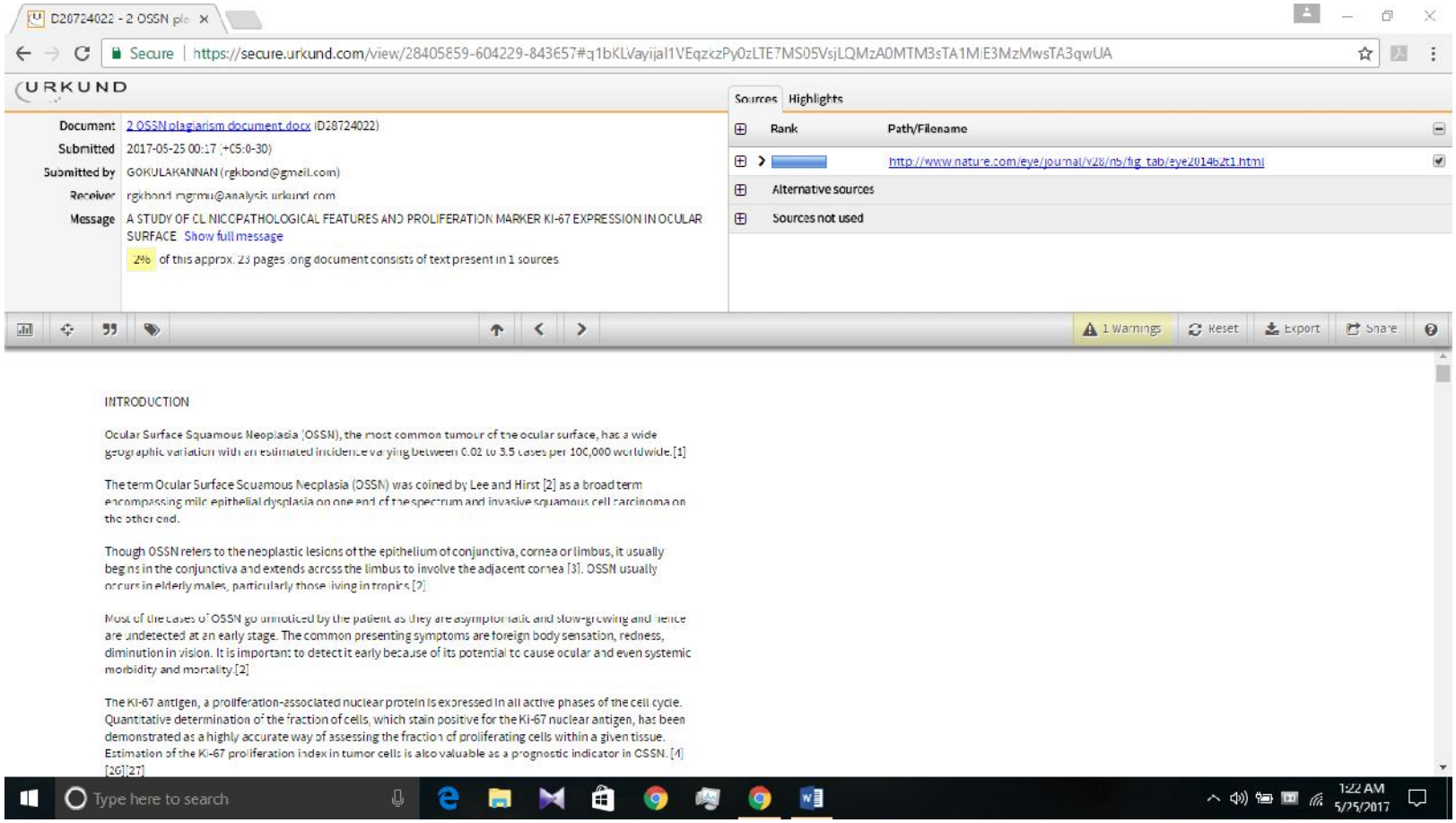
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INTRODUCTION
Ocular Surface Squamous Neoplasia (OSSN), the most common tumour of the ocular surface, has a wide geographic variation with an estimated incidence varying between 0.02 to 3.5 cases per 100,000 worldwide.[1]
The term Ocular Surface Squamous Neoplasia (OSSN) was coined by Lee and Hirst [2] as a broad term encompassing mild epithelial dysplasia on one end of the spectrum and invasive squamous cell carcinoma on the other end.
Though OSSN refers to the neoplastic lesions of the epithelium of conjunctiva, cornea or limbus, it usually begins in the conjunctiva and extends across the limbus to involve the adjacent cornea [3]. OSSN usually occurs in elderly males, particularly those living in tropics [2]
Most of the cases of OSSN go unnoticed by the patient as they are asymptomatic and slow-growing and hence are undetected at an early stage. The common presenting symptoms are foreign body sensation, redness, diminution in vision. It is important to detect it early because of its potential to cause ocular and even systemic morbidity and mortality.[2]
The KI-67 antigen, a proliferation-associated nuclear protein is expressed in all active phases of the cell cycle. Quantitative determination of the fraction of cells, which stain positive for the Ki-67 nuclear antigen, has been demonstrated as a highly accurate way of assessing the fraction of proliferating cells within a given tissue. Estimation of the Ki-67 proliferation index in tumor cells is also valuable as a prognostic indicator in OSSN. [4] [20][21]
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INTRODUCTION

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Most of the cases of OSSN go unnoticed by the patient as they are asymptomatic and slow-growing and hence are undetected at an early stage. The common

ABBREVIATIONS

OSSN	-	Ocular Surface Squamous Neoplasia
SCC	-	Squamous Cell Carcinoma
CIN	-	Conjunctival Intraepithelial Neoplasia
UV-B	-	UltraViolet-B
HIV	-	Human Immunodeficiency Virus
DNA	-	Deoxyribonucleic Acid
MIB 1	-	Methylation – inhibited binding protein 1
AJCC	-	American Joint Committee on Cancer
H&E	-	Hematoxylin and Eosin
MMC	-	Mitomycin-C
5FU	-	5 Fluorouracil

CONTENTS

INTRODUCTION

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The Ki-67 antigen, a proliferation-associated nuclear protein is expressed in all active phases of the cell cycle. Quantitative determination of the fraction of cells, which stain positive for the Ki-67 nuclear antigen, has been demonstrated as a highly accurate way of assessing the fraction of

proliferating cells within a given tissue. Estimation of the Ki-67 proliferation index in tumor cells is also valuable as a prognostic indicator in OSSN. [4][26][27]

This particular study was done to analyze the clinicopathogical characteristics of OSSN in a tertiary eye care center and to study the expression of proliferation marker Ki-67 in different grades of OSSN.

AIMS AND OBJECTIVES

This study is done

1) To analyze the clinical and histopathological characteristics of Ocular Surface Squamous Neoplasia (OSSN) cases, operated during a period of three years in a tertiary eye care center.
2) To analyze the association of clinical features with the histopathological type of OSSN.
3) To study the Ki-67 expression and evaluate its usefulness in Ocular Surface Squamous Neoplasia (OSSN) with respect to the histopathological grade.

REVIEW OF LITERATURE

ANATOMY AND HISTOLOGY OF OCULAR SURFACE:

Ocular surface consists of conjunctiva, cornea, and limbus.

CONJUNCTIVA:

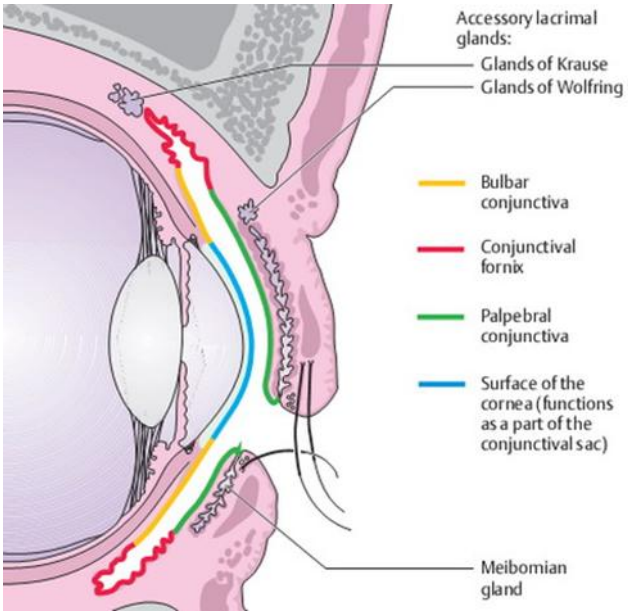
The conjunctiva is a transparent mucous membrane which lines the posterior surface of eyelids and anterior surface of the eyeball upto the limbus. The three subdivisions of the conjunctiva are: palpebral, forniceal and bulbar.[10]

The palpebral conjunctiva begins from the mucocutaneous junction of eyelid edge and lines the eyelid inner surface.

The forniceal conjunctiva is loose and freely mobile in the fornices.

The bulbar conjunctiva, covering the anterior aspect of sclera becomes continuous with the epithelium of cornea at the limbus.[10]

Figure showing parts of conjunctiva



HISTOLOGY OF CONJUNCTIVA

The conjunctival epithelium is non keratinizing stratified squamous epithelium. It is 5 cell layers thick and consists of basal cells, flattened polyhedral cells, superficial cells, along with goblet cells, lymphocytes and occasional dendritic melanocytes .

Figure showing10x view of normal conjunctiva

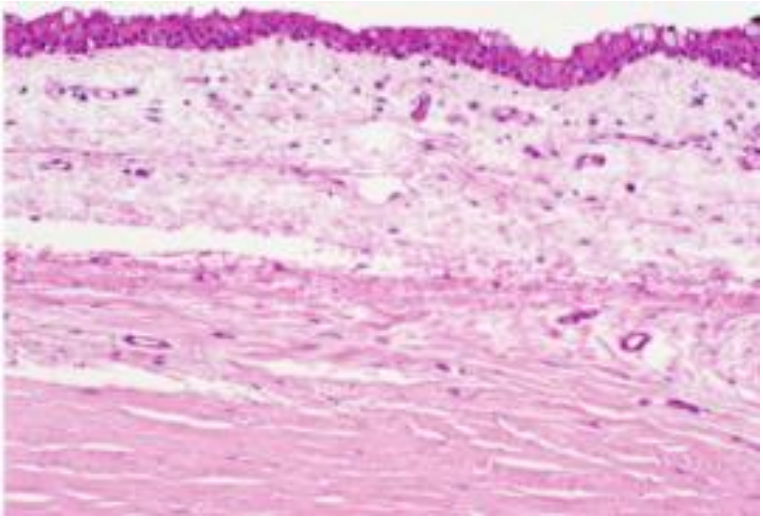
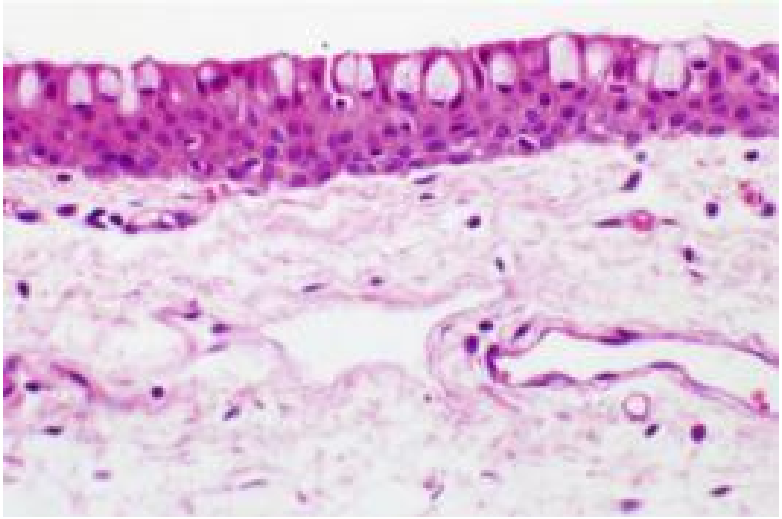


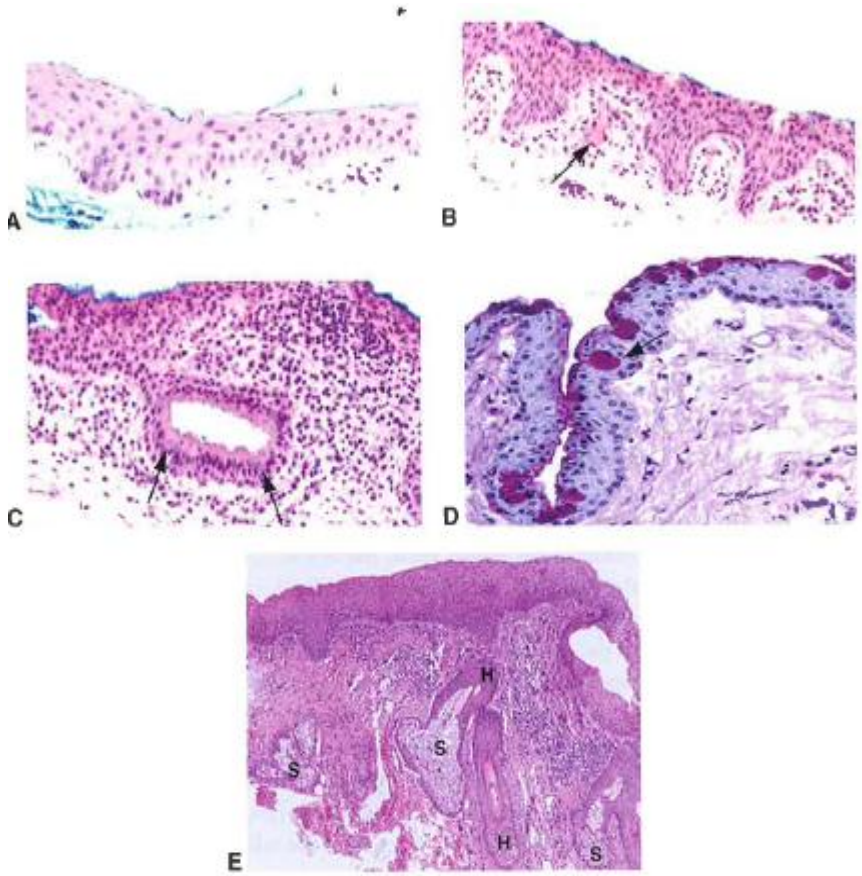
Figure showing 40x view of conjunctiva with intraepithelial goblet cells



The conjunctival stroma is made of loose connective tissue with numerous blood vessels along with lymphatics, nerves, accessory lacrimal glands, lymphocytes, plasma cells and few mast cells.[11]

The tear film plays an important role in maintaining the homeostasis of conjunctiva. Conjunctival goblet cells are involved in the production of mucinous portion of tear film. The tear film provides protection of corneal surface from microorganisms, foreign bodies and chemicals.[15]

Figure showing Histology of various subdivisions of conjunctiva:- A-epibulbar; B-palpebral; C-forniceal; D-Periodic acid-Schiff highlighting mucin in goblet cells; E-Caruncular conjunctiva with sebaceous glands.[11]



CORNEA

ANATOMY OF CORNEA:

Cornea is a specialized avascular transparent structure in the anterior wall of the globe, involved in refracting the light entering the eye and in providing structural integrity. The cornea measures 11 to 12 mm horizontally and 10-11 mm vertically. The cornea is 0.5 mm thick at the centre and 0.7 mm thick at the periphery.

The most important function of the cornea is to allow light to enter the eye and focuson the retina. The tear film covering the epithelium of the cornea is 7 μmin thickness and regulates the corneal epithelial activity.[15]

HISTOLOGY OF CORNEA:

The cornea is lined by non keratinizing stratified squamous epithelium, 5-7cell layers thick with a thin basement membrane. There are three different types of cells in corneal epithelium, namely superficial cells, wing cells and basal cells.

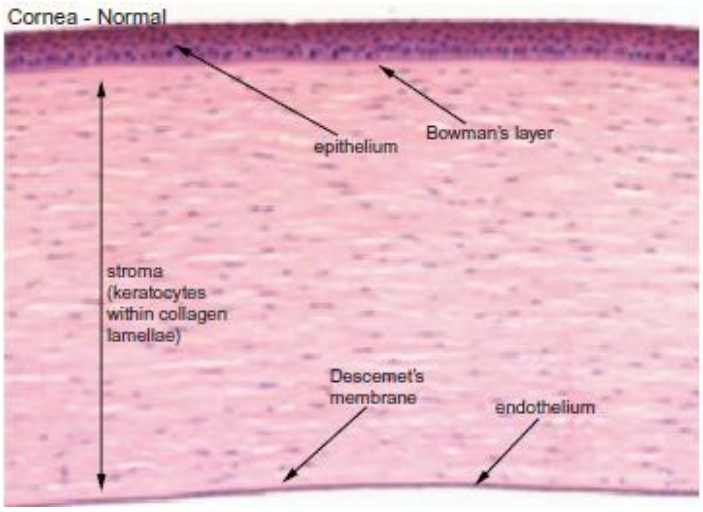
The superficial cells of cornea are flat with microvilli and microplicae and form two to four layers and do not show any mitotic activity.The wing cells of cornea are two to three layers thick. They have wing like processes and do not show mitotic activity.

The basal cells of the cornea are columnar forming a single layer and show mitotic activity unlike superficial and wing cells. The basal cells are attached to the basement membrane adjacent to bowman’s layer.[15]

The layers of cornea are as follows

1) Epithelium
2) Bowman's layer
3) Stroma
4) Descemet's membrane
5) Endothelium[10]

Figure showing Histology of cornea



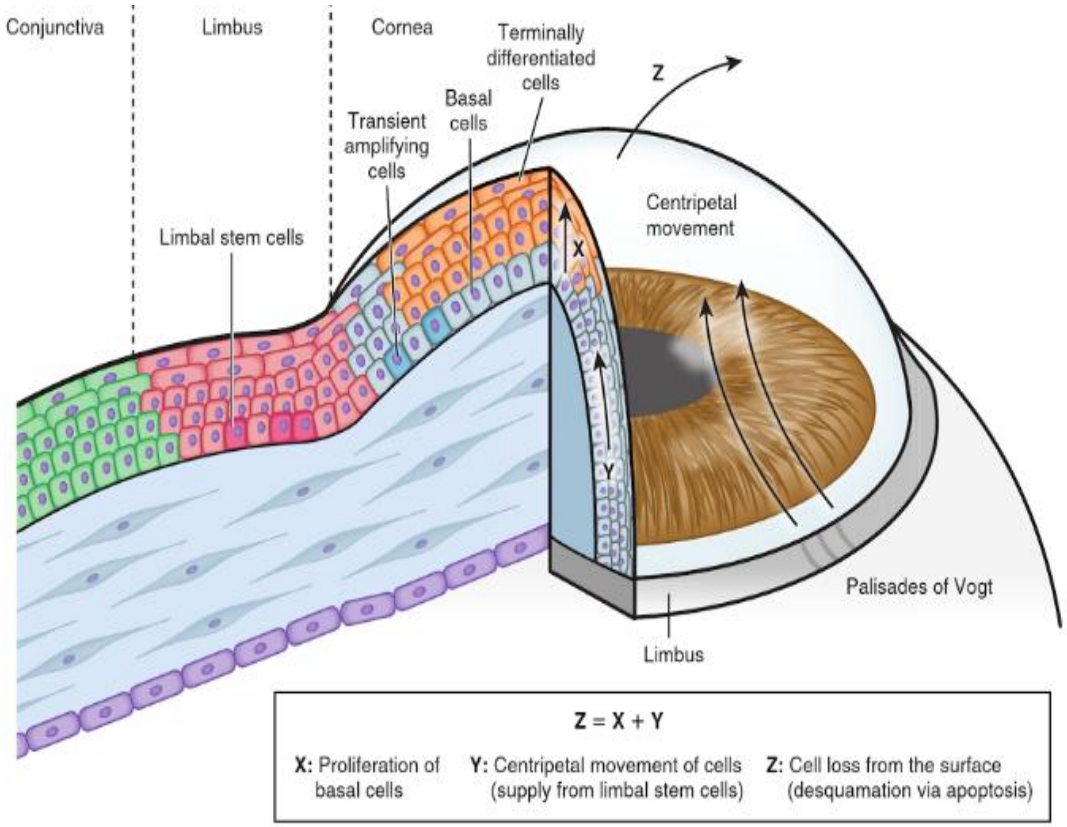
Corneal stroma is made of regularly spaced type 1 collagen to allow transparency of the cornea. Keratocytes, which are few in number are very important in maintaining the stromal structure. [15]

EMBRYOLOGY:

The conjunctival epithelium and limbal epithelium are derived from Surface ectoderm.

The corneal epithelium is derived from surface ectoderm. The stroma is derived from mesenchyme. The keratocytes and corneal endothelium is derived from neural crest cells[15]

Figure showing the Lineage of corneal epithelial cells[15]



LIMBUS

The term ‘limbus’ meaning border, refers to the border zone between transparent cornea and the opaque sclera. Limbus is not a distinct tissue but only a zone formed by the junction of the epithelium of cornea and conjunctiva, thereby separating cornea, conjunctiva, sclera and uvea. The limbus is elliptical in shape with horizontal orientation of long axis.[13][39]

Histologically a limbal tissue can be represented anteriorly by a line between the peripheral extremity of Bowman's and Descemet's membrane and posteriorly by a line from scleral spur perpendicular to the tangent of the

external surface of the globe. The limbus consists of stratified squamous epithelium and loose connective tissue, resembling conjunctival histology. The collagen of cornea is little less eosinophilic than that of conjunctiva in histological sections and it is difficult to clearly establish the demarcation by conventional staining methods.[39]

The limbus is of considerable interest because it maintains the nutrition of the cornea in the periphery. It has the pathways for the outflow of aqueous humour. Limbus also is the site of surgical incisions for cataract surgeries[39]

The ocular surface is made up of constantly restoring epithelial cells, which are renewed by proliferation of a specific group of cells known as Stem cells.The stem cells of the cornea are present in the basal layer of the limbus. The conjunctival stem cells are evenly distributed in the bulbar surface and also present in the fornices.[13]

The normal limbus protects against vascularization of the cornea by the conjunctival blood vessels. The limbal stem cells are frequently associated with tumorigenesis of OSSN. Some studies have reported that the mutated limbal stem cells lead to disorganized cell growth. The stem cells of the limbus, once affected, may lead to migration of conjunctival cells over the corneal surface and subsequent neovascularization. However, the exact mechanism of origin of OSSN is yet to be ascertained. [13]

Figure showing conjunctival (1), limbal (2), corneal (3) epithelium and Bowman's membrane(4)

OCULAR SURFACE SQUAMOUS NEOPLASIA

Ocular surface tumors have wide range from benign non-neoplastic lesions to highly invasive and aggressive malignancies.[12]

Von Graefe described the first case of Ocular Surface Squamous Neoplasia (OSSN) as early as 1860. Lee and Hirst coined the term Ocular Surface Squamous Neoplasia in 1995 to encompass a wide spectrum of neoplastic lesions affecting the conjunctiva and cornea[2]

CLASSIFICATION OF OSSN

Ocular Surface Squamous Neoplasia (OSSN) has three grades[3] :

1. Benign dysplasia
    - Papilloma
    - Pseudotheliomatous hyperplasia
    - Benign hereditary intraepithelial dyskeratosis

2. Preinvasive OSSN
    - Conjunctival Intraepithelial Neoplasia/carcinoma in situ
3. Invasive OSSN
    - Squamous carcinoma
    - Mucoepidermoid carcinoma

EPIDEMIOLOGY:

Ocular Surface Squamous Neoplasia (OSSN), has a wide geographic variation with an estimated incidence varying between 0.02 to 3.5 cases per 100,000 worldwide.[1] OSSN is the most common primary ocular surface neoplasm.[12] In the later decades of life, OSSN is the third most common tumor of the ocular region after Melanoma and Lymphoma.[1]

OSSN is very common in tropical regions and decreases in incidence as we move towards temperate regions. There is an increasing trend of OSSN in developing nations with high prevalence of HIV infection. Dark skinned Caucasians are the most common group of people affected [3].

Most of the cases are undetected as they are usually asymptomatic and slow growing. Males are most commonly affected as they are more inclined to be exposed to solar radiation due to outdoor activity.[2]

OSSN is more common in advanced age groups, the average age of presentation is in sixth decade around 56 years. However the incidence of preinvasive OSSN (conjunctival intraepithelial neoplasia) is noted in individuals 5-9 years younger than average age of onset of invasive OSSN,

which may be attributed to the progression of the tumor from pre-invasive neoplasia towards invasive squamous cell carcinoma[2].

ETIOPATHOGENESIS OF OSSN:

The definite pathogenesis of OSSN is still not known, however many risk factors are implicated in the development and progression of OSSN.

1) Ultraviolet B Radiation :

Sunlight exposure is one of the most important risk factors in the pathogenesis of OSSN. This can be attributed to the increased incidence of OSSN in people living in the tropics, dark skinned individuals, male gender (exposed to more outdoor occupation and activity than females) and in persons with pre-existing actinic skin lesions.

Histological features of solar injury which is considered as a major risk factor, is found in more than half of the cases of OSSN. Ultraviolet B radiation results in damage to nucleotide excision repair and the resultant DNA repair is associated with OSSN. UV-B radiation is also implicated in causing p53 (a tumor suppressor protein regulating cell cycle) mutation which is closely associated with OSSN[3],[4]

2) Xeroderma Pigmentosum :

In Xeroderma Pigmentosum, there is defective DNA repair mechanism. It is an autosomal recessive disorder implicated in predisposition to aggressive cases of OSSN, especially in cases with increased exposure to sunlight. Also it is associated with early age of onset of OSSN.[3]

3) Human Papilloma Virus Infection

HPV genotypes 6 and 11 are associated with both preinvasive as well as invasive OSSN. DNA of HPV 16 and 18 are seen in conjunctival intraepithelial neoplasia and invasive OSSN. The possibility of HPV and UV-B radiation together involved in oncogenesis of OSSN is more likely.

4) Human Immunodeficiency Virus

HIV is emerging as one of the important risk factors in the development of OSSN. HIV associated OSSN is much common in African population [10]. OSSN tends to occur in an earlier age in HIV affected individuals . OSSN follows a much aggressive course in HIV affected individuals, in comparision with normal individuals. Also invasive OSSN is more likely in HIV infected , and may require orbital exenteration in such cases[3]

5) Chemical Exposure

Exposure to beryllium, arsenic, trifluridine, petroleum products is also associated with OSSN.

6) Cigarette Smoking :

Heavy cigarette smoking is an important risk factor in the development and progression of OSSN, as smoking reduces the quality and amount of tear film produced, thereby predisposing to squamous metaplasia.

Other risk factors associated with OSSN are

7) Injury to Ocular Surface

8) Vitamin A deficiency

9) Herpes simplex virus type 1 infection

10) **Immunosuppression due to other malignancies like lymphoma, organ transplantation**

11) **Exposure to dust**

12) **History of squamous cell carcinoma of the skin of head and neck**[12]

The pathogenesis of OSSN is probably due to disorderedepithelial maturation induced by irritants. Various studieshave not shown any consistent genetic abnormalities or mutations in tumor cells.[15]

Beta 2 microglobulins and HLA class I antigens are reduced in mild to moderate dysplasia and are absent in carcinoma in situ and invasive carcinomas. This implies the possible role of cytotoxic T lymphocytes in development of noninvasive OSSN. HLA class II antigens are not expressed ininvasive OSSN, thereby implying the possible role of helper T lymphocytes in its pathogenesis[15]

CLINICAL FEATURES OF OSSN :

OSSN is usually unilateral and presents as an elevated lesion in the interpalpebral region near the corneoscleral limbus, either temporally or nasally. Bulbar conjunctiva is involved most commonly, palpebral conjunctiva is affected rarely. OSSN presents as a grey white conjunctival growth, at or near the limbus, with a characteristic blood vessel tufts in the interpalpebral area. It can also become fleshy if there are dilated feeder vessels. OSSN presents either as diffuse lesion or well defined lesion. The affected corneal region is also slightly elevated.

It is very difficult to clinically distinguish preinvasive OSSN from Invasive SCC. The presence of extensive leukoplakia, feeder vessels, larger size favours malignancy. Nodular lesion also favours SCC. Diffuse ill-defined lesions of OSSN may sometimes mimic as a case of chronic conjunctivitis.

OSSN is usually asymptomatic as it is a slowly growing tumour. It can also present with symptoms of chronic irritation, redness, foreign body sensation, decrease in vision.

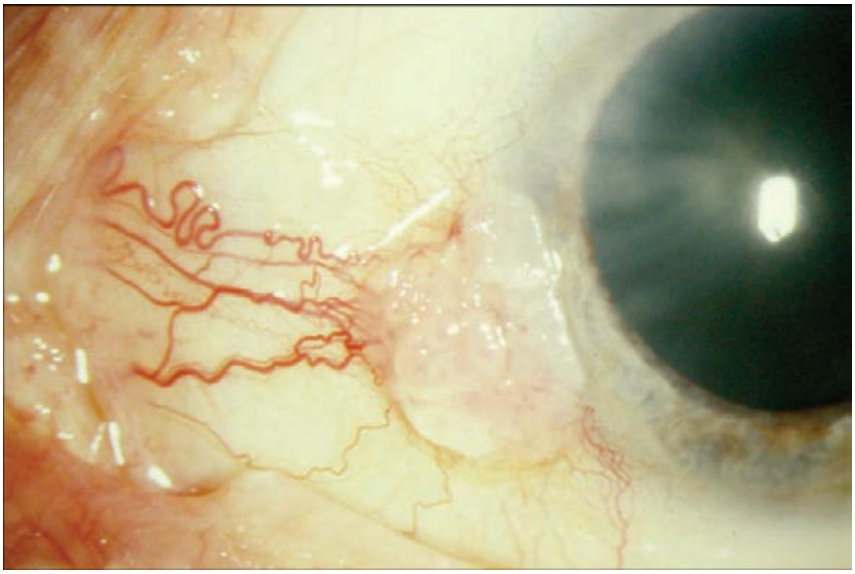
OSSN rarely metastasise since it is a low grade malignancy. However preauricular, submandibular and deep cervical nodes may be enlarged in a few cases.

MORPHOLOGICAL TYPES OF OSSN:

CONJUNCTIVAL OSSN :

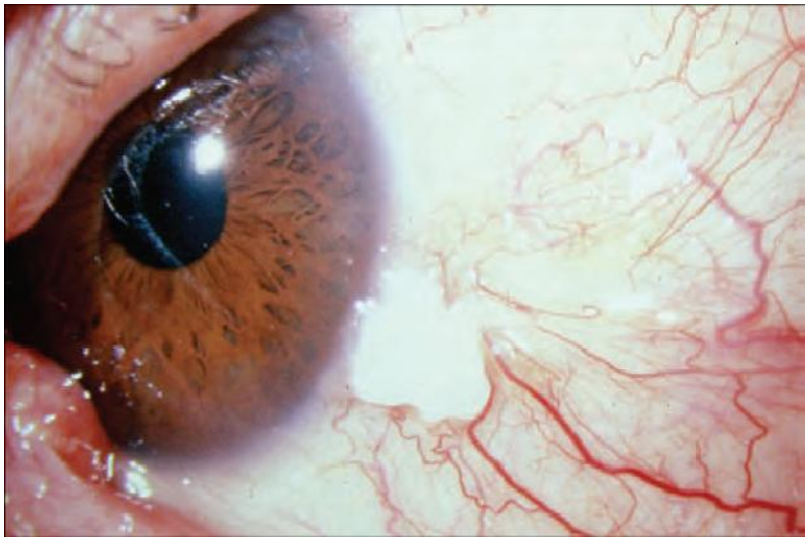
Gelatinous: Well defined gelatinous lesions are the commonest type can present as circumscribed nodular lesions, and are associated with aggressive clinical course and increased risk of metastasis to the adjacent lymph nodes. The diffuse type is less common and presents as ill-defined growth, or redness of conjunctiva similar to chronic conjunctivitis.

Figure showing Gelatinous type of Squamous cell carcinoma presenting as a translucent mass in the limbus[12]



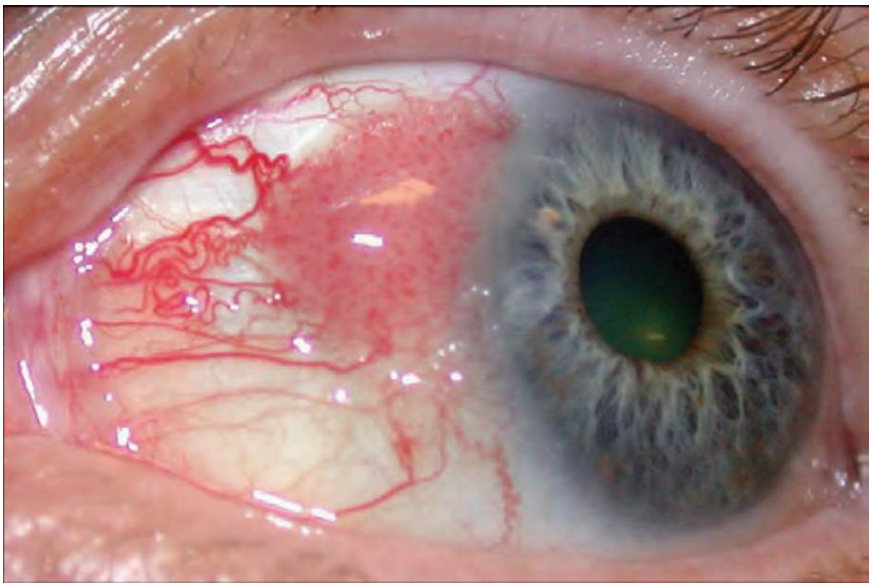
Leukoplakic : This type of OSSN usually presents as a superficial , opaque-white, focal thickening of the ocular surface epithelium . Leukoplakic type of OSSN is usually pre-invasive.

Figure showing Leukoplakic squamous cell carcinoma of conjunctiva - opaque white hyperkeratotic plaque[12]



Papilliform: The papillomatous type appears as an exophytic soft tissue lesion, which is highly vascularized.

Figure showing Papillomatous squamous cell carcinoma – pinkish conjunctival growth with numerous dilated blood vessels [12]



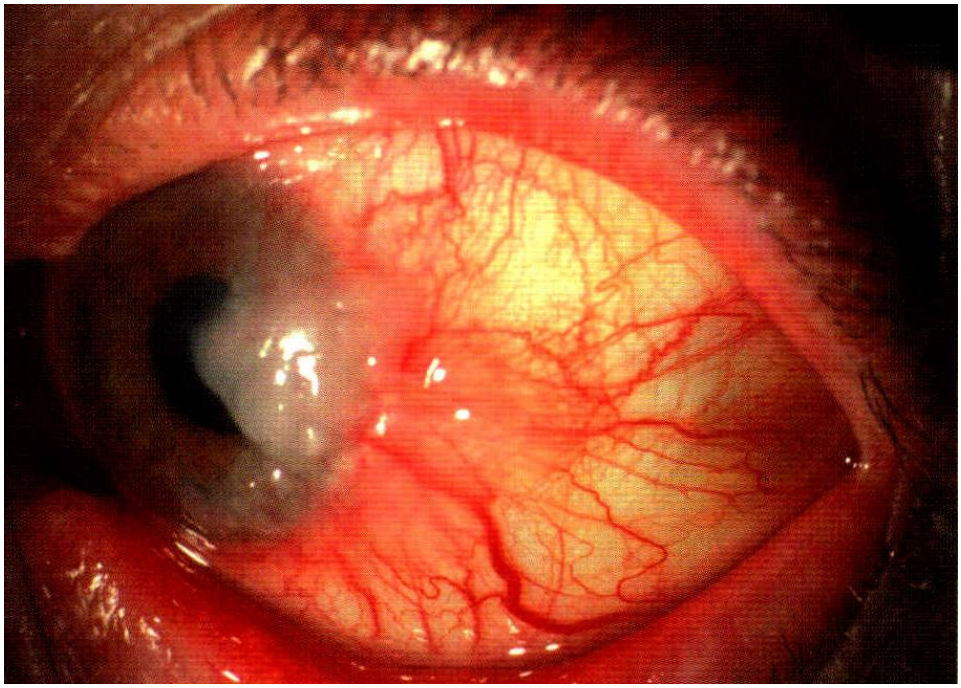
The size of the lesion, usually correlates with the malignant histology. But it is difficult to classify OSSN, based on morphological appearance as benign, preinvasive or invasive malignancy. Features like spontaneous bleeding, increased vascularity of lesion, intraocular invasion points towards a malignant type of OSSN.[2],[3],[12]

There is a marked corkscrew vascular pattern in OSSN. Though surface keratinization is not specific for OSSN, any ocular surface lesion with surface keratinization should be carefully evaluated.[10]

CORNEAL OSSN

Corneal OSSN lesions present as mottled ground glass sheet like appearance. They appear as slightly elevated, well defined, grey white lesions which are commonly avascular.

Figure showing Invasive OSSN with corneal involvement[10]



The corneal lesions are asymptomatic and slow growing. They usually turn out to be pre-invasive OSSN on histopathological examination and carry increased risk of recurrence.

CLINICAL DIAGNOSIS AND EVALUATION

CLINICAL EXAMINATION

Patients with a suspected tumor of the ocular surface need a comprehensive ophthalmologic evaluation which includes the clinical appearance of the lesion, size, location and assessing the lesion at the slit lamp, to look for invasive features, if any.

SLIT LAMP ASSESSMENT

Slit lamp examination is part of clinical evaluation which is used to identify the type of lesion, its appearance, location, to measure the dimensions of the lesion, and conjunctival vascularity

ULTRASOUND BIOMICROSCOPY (UBM)

This is particularly helpful if corneal orscleral invasion is suspected, as it useful in imaging of deeper ocular structures.

ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY (ASOCT)

This is an important and recent diagnostic modality for evaluation of histomorphological features in cases of ocular tumours.

CONFOCAL MICROSCOPY

Confocal Microscopy is an important tool in the diagnosis, treatment and follow up of OSSN. it is done as an out-patient procedure and aids in thepathological diagnosis of OSSN. Confocal microscopy also helps to differentiate between carcinoma in situ and invasive carcinomas. It also is useful in follow up of OSSN cases, by assessing the recurrence, and in evaluating the response to chemotherapy.[3],[20]

CYTOLOGY

The maturation of tumour cells in OSSN is abnormal and disorganized and the study of morphological features of the desquamating ocular surface cells by cytology forms an important baseline investigation.

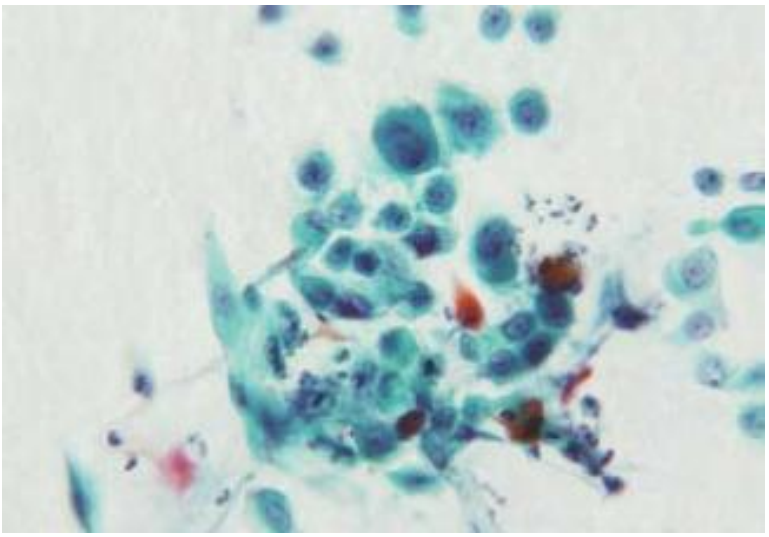
The cytological features of dysplastic epithelial cells are pleomorphism, with enlarged hyperchromatic nuclei with irregular nuclear outline, prominent nucleoli, increased nucleocytoplasmic ratio and also increased mitotic figures are also seen.

Desquamating cells of the ocular surface are studied by two methods namely

1) Exfoliative cytology in which tumourcells using a cytobrush or a platinum spatula
2) Impression cytology using Biopore membrane or cellulose acteate papers

Biopore membrane is preferred nowadays due to advantages of better cell adherence and storage for further analysis.

Figure showing Impression cytology of the ocular surface showing dysplastic squamous cells with increased nucleus:cytoplasmic ratio, hyperchromatic nuclei, irregular nuclear membranes, and prominent nucleoli



ADVANTAGES

1) Easy to perform
2) Non invasive
3) Useful in differentiating between benign and malignant cells
4) Helpful in detection and follow up of cases after treatment, chemotherapy and in cases of recurrence.

DISADVANTAGES

1) Mild discomfort to the patient
2) Depth of invasion cannot be assessed
3) Cannot be used to differentiate between cases of carcinoma in situ and invasive carcinoma
4) False negative results may arise due to improper sampling.

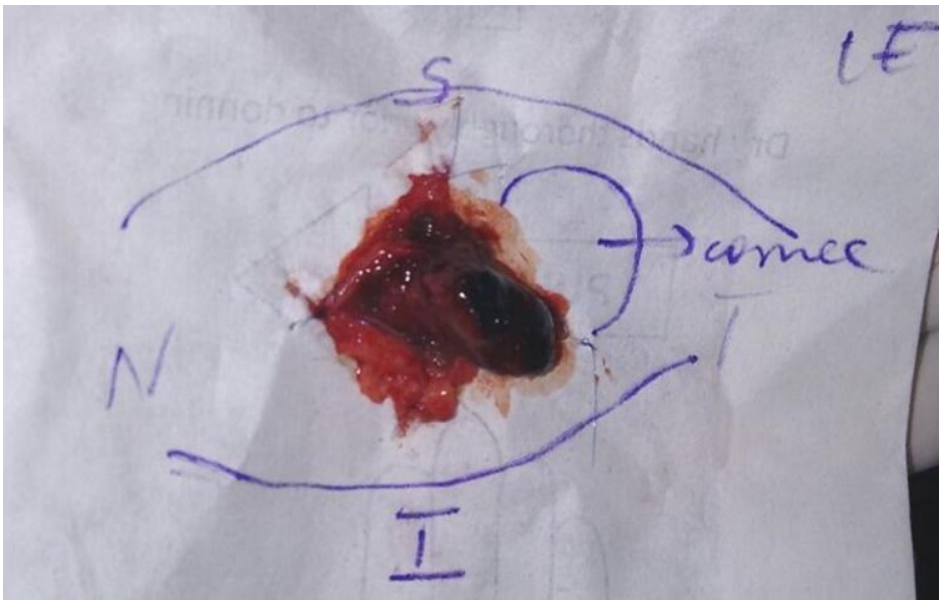
HISTOPATHOLOGY OF OSSN :

A high degree of suspicion is needed in any case with discrete thickening of the conjunctiva or cornea, especially if associated with prominentconjunctival vasculature and it should probably be excised or biopsied. Surgical excision is most common procedure done. It is both diagnostic and also curative in most cases of OSSN.

Figure showing Squamous cell carcinoma invading the limbus and the anterior chamber [11]

After surgery, the excised lesion is placed over a filter paper along with figure representing the exact site of lesion, laterality, with or without margins. The excised lesion along with filter paper is sent to the histopathology laboratory in 10% neutral buffered formalin. Margins should be submitted after mentioning them as medial/lateral/superior/inferior and then processed in separate cassettes.[4],[11],[20]

Figure showing method of submission and pictoral documentation of excised ocular surface lesion, placed on filter paper



Histopathological evaluation is the mainstay in diagnosing OSSN after incisional or excisional biopsy.[20]

The histological features seen in OSSN are hyperplasia of the stratified squamous epithelium, loss of goblet cells, loss of polarity, nests or sheet like pattern of arrangement of neoplastic cells, pleomorphic and hyperchromatic nuclei, increased nucleo-cytoplasmic ratio, mitotic figures and chronic inflammatory cell infiltrate.

The most significant histopathological assessment to be made in OSSN is to assess microscopically, if the tumour is contained within the basement membrane or the tumour cells have traversed the epithelial basement membrane and invaded into the stroma.

HPV associated lesions show koilocytic change. Koilocytes are mature squamous cells with dense eosinophilic cytoplasm and a distinct halo around the enlarged nucleus.

The term Conjunctival intraepithelial neoplasia (CIN) is used to describe lesions limited by the basement membrane. However, the term CIN is not commonly used nowadays as it is not possible to determine stromal invasion on clinical examination, so the term OSSN is preferred. CIN is to be regarded more of a histologic term denoting only noninvasive lesions.

PREINVASIVE OSSN

Preinvasive OSSN is graded as mild, moderate and severe based on extent of epithelial dysplasia [4],[11],[20]

(i) Mild – CIN grade I : dysplasia involving lower third of the epithelium

(ii) Moderate – CIN grade II : dysplasia extending into the middle third of the epithelium

(iii) Severe – CIN grade III : dysplasia involving whole thickness of epithelium with intact basement membrane – also called as carcinoma in situ . There is also total loss of normal cellular polarity

Figure showing Conjunctival intraepithelial neoplasia with abrupt transition from normal epithelium (on the right) to the tumour (on the left)

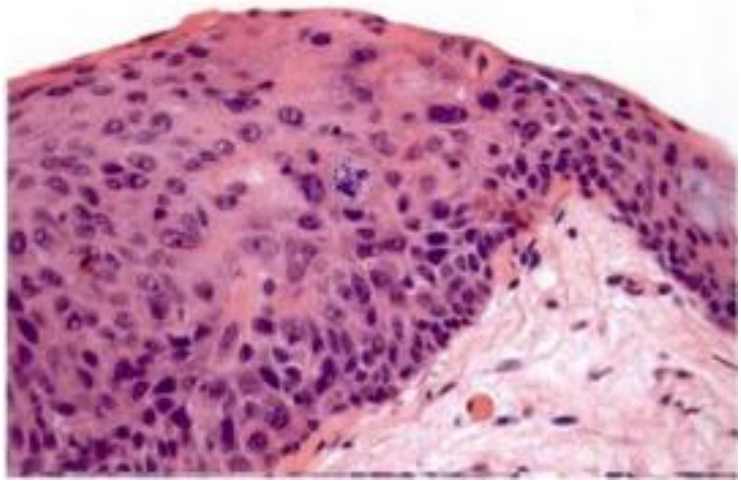
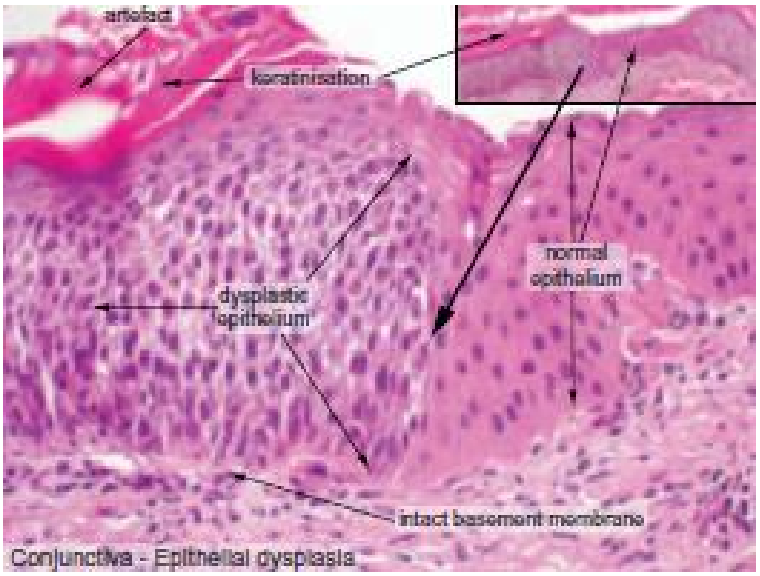
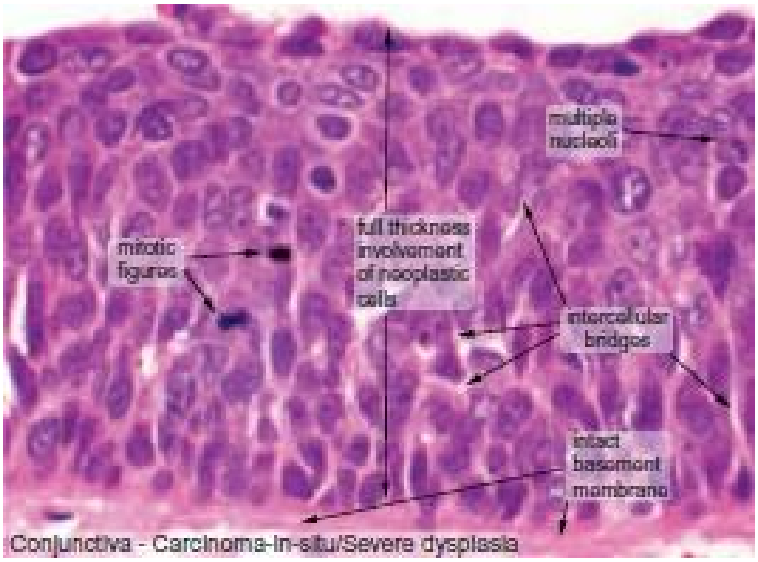


Figure showing CIN II: moderate epithelial dysplasia



Complete involvement of whole epithelium without any damage to basement membrane is the feature of Conjunctival carcinoma in situ

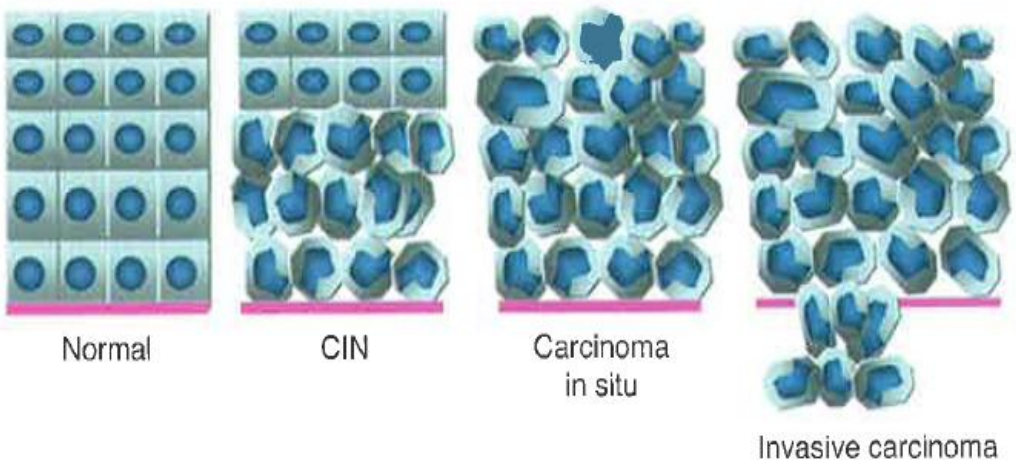
Fig (18) CIN III: conjunctival carcinoma-in-situ



INVASIVE OSSN

When the basement membrane is breached , the neoplastic cells invade into the stroma, the term carcinoma is used.

Figure representing the progression of OSSN [11]



Invasive OSSN is characterized by nests, cords, and sheets of tumour cells infiltrating into the stroma. Individual tumour cells are large, polygonal with moderate to scant cytoplasm and hyperchromatic nuclei.

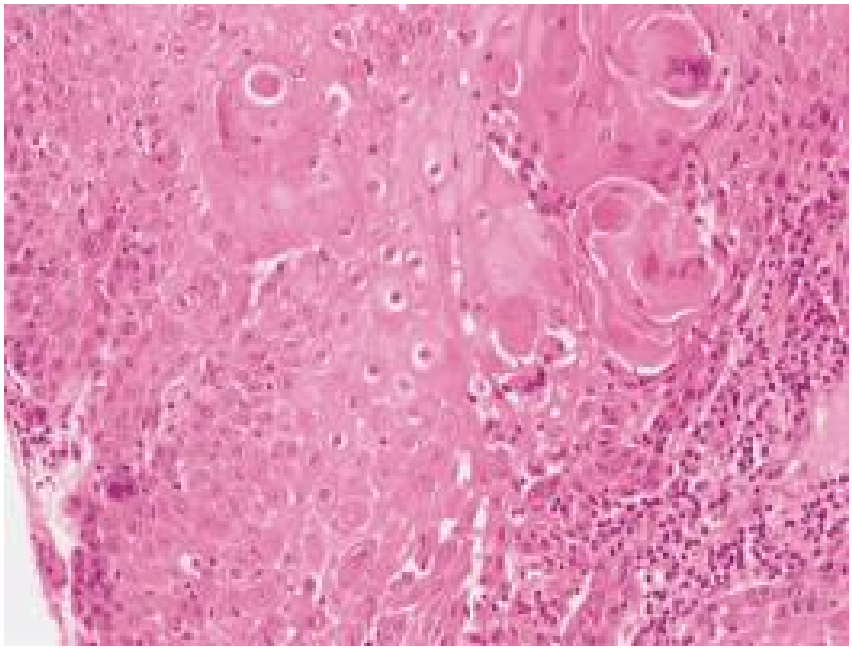
1.SQUAMOUS CARCINOMA :

Squamous cell carcinoma is classified based on degree of keratinization into well differentiated , moderately differentiated and poorly differentiated.

WELL DIFFERENTIATED SCC :

Well differentiated carcinomas show varying degree of cellular pleomorphism, hyperchromatic nuclei, prominent nucleoli, moderate to abundant eosinophilic cytoplasm, few mitotic figures and well developed keratinization

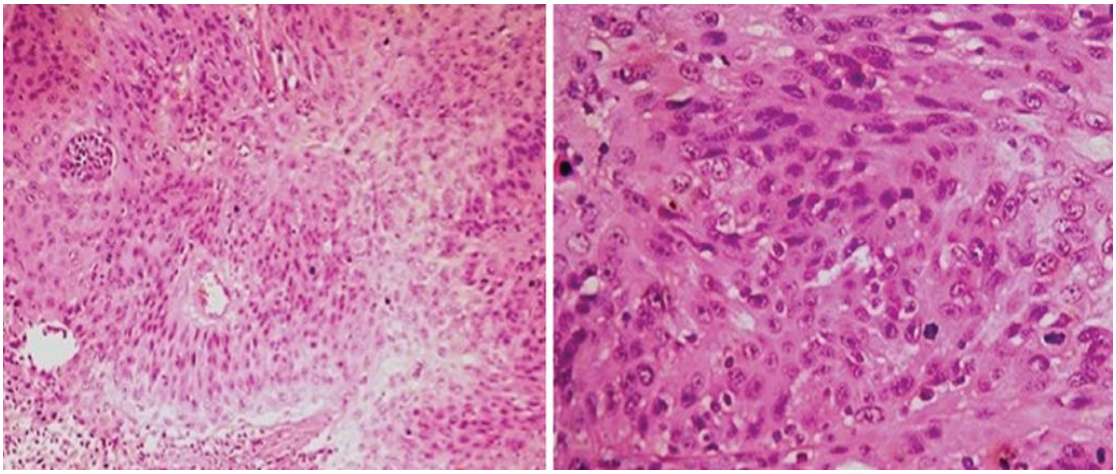
Figure showing well differentiated squamous cell carcinoma of conjunctiva with atypical squamous cells and increased keratin pearl formation.



MODERATELY DIFFERENTIATED SCC:

Moderately differentiated carcinomas show little less keratinization as compared to well differentiated ones, and the tumour cells show more pleomorphism along with features which are between well differentiated and poorly differentiated SCC.

Figure showing low power (left image) and magnified high power (right image) view of invasive OSSN (Moderately differentiated SCC)



POORLY DIFFERENTIATED SCC:

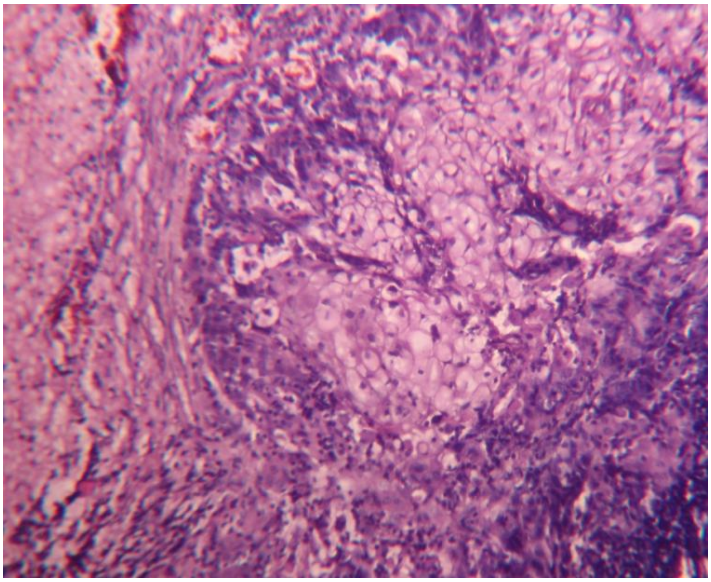
Poorly differentiated squamous carcinoma show more immature tumour cells with scant cytoplasm, more nuclear pleomorphism, bizarre tumour cells, giant cells, minimal or absent keratinization and increased atypical mitoses. They are less common and more aggressive.

2.MUCOEPIDERMOID CARCINOMA

Mucoepidermoid carcinoma is a rare type of invasive OSSN. Mucoepidermoid carcinoma may be similar to that of squamous cell carcinoma admixed with mucin producing cells, intermediate cells.

It is a highly aggressive tumour with higher rates of recurrence and intraocular spread, local spread as well as distant metastasis is rare or occurs very late. So extended follow up is required in these cases

Figure showing 10x view of mucoepidermoid carcinoma with admixture of dysplastic squamous cells, mucinouscells, few clear cells and intermediate cells



RARE VARIANTS

Spindle cell carcinoma, Papillary Squamous cell carcinoma, Acantholytic squamous cell carcinoma are the uncommon types of Squamous cell carcinoma reported [20]

ELECTRON MICROSCOPY

Electron microscopy in OSSN show the following findings –increased number of mitochondria, tonofilaments and endoplasmic reticulum; reduced desmosomes, altered basement membrane and granular material deposition between the basement membrane and bowman layer[4]

DIFFERENTIAL DIAGNOSIS :

OSSN may be clinically confused with a lot of other conjunctival lesions. The differential diagnosis for OSSN are

1) Pterygium
2) Dermoid
3) Pinguecula
4) Choristoma
5) Atypical conjunctival papilloma
6) Actinic keratosis
7) Keratoacanthoma
8) Pyogenic granuloma
9) Pseudo-epitheliomatous hyperplasia
10) Amelanotic nevus
11) Malignant melanoma
12) Lymphoma

METASTASIS AND OSSN :

Metastasis of OSSN though rare is observed in a few cases of advanced lesions andin highly invasive and aggressive tumours like mucoepidermoid carcinoma. During the early stages of the disease, the signs of local spread are not obvious.

Features indicative of ocular and orbital extension are anterior chamber reaction, circumciliary injection, undetected sclerocorneal perforation, development of peripheral anterior synechiae which may be found during the early stages.

AIDS patients with associated invasive OSSN also have an increased risk of metastasis.

Regional lymph node metastasis is observed in a few cases of malignant OSSN. Preauricular group of lymph nodes are involved early in tumours which are located in temporal limbal region. Tumours involving the nasal limbal region metastasize commonly to the anterior cervical lymph nodes. Metastasis to the parotid, longs and bone are also rarely noted.[12],[17]

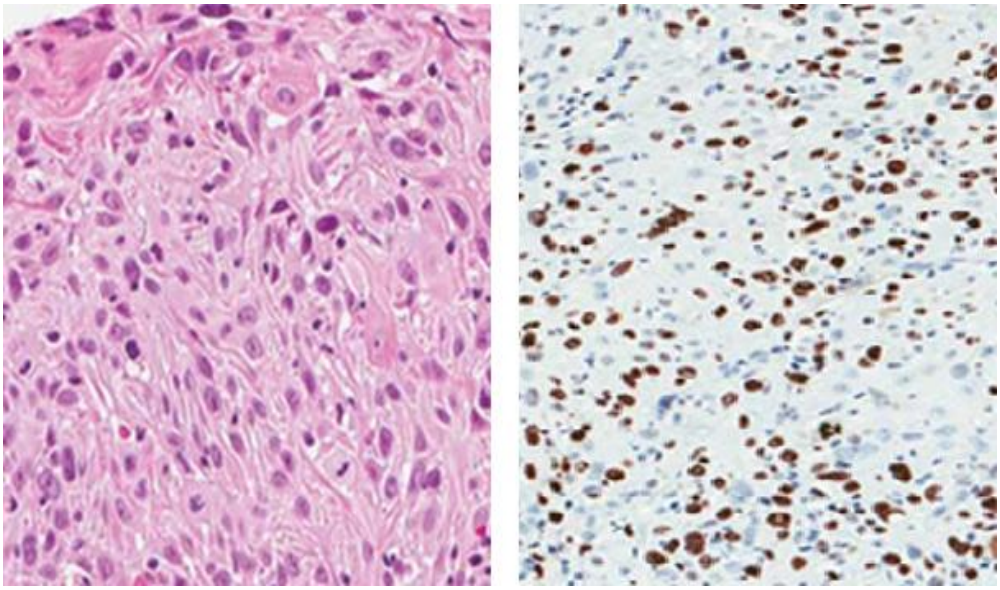
ROLE OF Ki-67 IMMUNOHISTOCHEMISTRY IN OSSN :

The Ki-67 antigen was identified in the 1980s with the help of a monoclonal antibody used against nuclear antigen from a Hodgkin lymphoma derived cell line. Ki-67 is named after Kiel university, Germany, where it was first identified ; the label 67 indicates the clone number on the 96 well plate. [24],[25]. Ki-67 is a non-histone protein. The location of Ki-67 gene is human chromosome 10 (10q25)[27]

There is wide variation in the expression of Ki-67 in different cell cycle phases. While Ki-67 is expressed at low levels in G1 and S phases, the level of Ki-67 increases to high values in early mitosis and Ki-67 levels significantly drops in late mitosis. Ki-67 is expressed by proliferating cells in all active stages of cell cycle (G(1), S, G(2), and mitosis), except resting cells G(0), thus making human Ki-67 expression as an excellent marker for determining growth fraction of a given cell population. Since the cellular proliferation is closely linked to tumor recurrence, Ki-67 is considered as a potential molecular indicator in the prognosis of a tumour[26],[27]

Ki-67 levels on paraffin sections are done most commonly using MIB-1 antibody[24].Staining is nuclear (usually nucleolar or perinucleolar) and can be diffuse or granular or mix of both[26]

Figure showing H&E stained conjunctival squamous cell carcinoma (left) and Ki-67 stained areas of the conjunctival tumour (right)



The Ki-67 marker expression is used as an important indicator in diagnosis and prognosis of various malignancies.[6] Ki-67 expression in carcinomas of the prostateand the breast are done extensively and in these tumors, the prognosis and recurrence have been repeatedly showed close correlation with Ki-67 values [26]. Certain studies have shown that Ki-67 index can be used as a prognostic marker for OSSN.[5],[6]

The percentage of Ki-67-positive tumour cells (known as the Ki-67 labeling index) often correlates with the clinical course of the disease[26],[27].

TREATMENT:

Treatment of OSSN varies from complete surgical excision of well delineated tumors to topical chemotherapy in larger unresectable lesions. In case of squamous cell carcinomas of ocular surface, the surgical procedure done, may vary from Excision biopsy to Orbital exenteration.

Factors in determining the mode of treatment are

1) Size including depth of the lesion
2) Clinical invasiveness
3) Status of other eye
4) Age of the patient
5) Associated co-morbidities

SURGICAL MANAGEMENT

Complete surgical excision with sufficient margin clearance is the usual treatment of choice. ‘No touch, wide margin’ technique is currently the preferred form of surgical excision. Incisional biopsy is done for large ill defined lesions.

If the cornea is significantly involved, alcohol assisted keratoepitheliectomy is preferred. Lamellar sclerokeratoconjunctivectomy is indicated in some cases of invasive OSSN (especially those lesions which are firmly adherent), so as to enable complete removal of the tumour.[20]

Enucleation is done when a malignant OSSN shows features of intraocular invasion through the cornea or sclera.

Exenteration is usually done in advanced cases of malignant OSSN invading into the anterior orbit. Though Exenteration is useful in eliminating the residual tumour, failure is not uncommon with this procedure.[12]

CRYOTHERAPY :

Cryotherapy is an important procedure, done to the tissue adjacent to and immediately beneath the lesion. It is done either along with the surgical excision or in the early postoperative period. Cryotherapy is done in the early post-operative period, if the surgical margins of the excised lesion are histopatholgically involved by the tumour.

Cryotherapy is important in reducing the recurrence of OSSN. However, localized tissue swelling, reduced ocular motility, scleral melting are a few disadvantages of this procedure [12]

MEDICAL MANAGEMENT

CHEMOTHERAPY

Topical and intralesional chemotherapy is a simple and cheap treatment modality, used in OSSN. Chemotherapy is particularly useful in diffuse lesions with indistinguishable margins, recurrent OSSN, positive margins after surgical excision and in large tumours as neoadjuvant chemotherapy prior to surgery.

Advantages

1) It treats the entire ocular surface, thereby averts the need for clear tumour margins.
2) It also reduces the chances of limbal stem cell deficiency
3) Cost effective

Disadvantages

1) Penetration into larger tumours is limited
2) Side effects of individual chemotherapy drugs to ocular surface and nasopharyngeal epithelium.

MITOMYCIN-C (MMC)

MMC is an anti-tumour drug that inhibits DNA synthesis in G1 and S phases of cell cycle. MMC causes cell death in cases of OSSN by apoptosis and necrosis.

MMC is used in dose of 0.02-0.04% , four times a day , 1 week on and 1 week off treatment, for 4 cycles. The drug free period is for repair and regeneration of healthy cells of ocular surface and to avoid the serious side effects of MMC therapy

Adverse Effects of MMC are redness, sclerocorneal erosions and ulcers, punctate epithelial keratopathy, limbal stem cell deficiency, tissue necrosis , dry eye, uveitis, cataract and glaucoma[4]

5-FLUOROURACIL (5FU)

5FU is an antimetabolite which acts during the S phase of cell cycle. The mechanism of action is by prevention of DNA and RNA synthesis

5FU is given in dose 1%, four times a day, 1 week on and 1 month off treatment, the benefit being better efficacy and tolerance.

Adverse effects of 5FU are similar to that of MMC, but less common due to the longer drug free interval between cycles[4]

IMMUNOTHERAPY

Immunotherapy is one of the latest treatment modalities used in the treatment of OSSN. Interferon alpha 2b (IFN-α2b) is a low molecular weight glycoprotein, produced by leukocytes. It attaches to the cell surface receptors, thereby inhibiting intracellular events and producing antineoplastic and antiviral properties.

ADVANTAGES :

1) Can be used in recurrent cases
2) Can be used in Chemotherapy resistant OSSN
3) Can be used to treat small multiple lesions

DISADVANTAGES :

1) Costly
2) Restricted availability in select specialized compounding pharmacies
3) More toxic than Chemotherapy

Mode of administration of IFN-α2b is topical drops or subconjunctival injections or intralesionalinjections. The dose recommended is 1million and 3 million IU/ml, applied four times a day as topical drops.

Adverse effects of IFN-α2b are flu like symptoms and irritation over the ocular surface

PEGYLATED INTERFERON ALPHA 2B

Pegylated Interferon alpha 2b, though rarely used, is derived from recombinant interferon alpha 2b is less toxic and in some cases more effective in treatment of OSSN

OTHER DRUGS

Topical and sub-conjunctival injections of anti- vascular endothelial growth factor (VEGF) shows good results in the treatment ofOSSN.[28]

Topical Bevacizumab also shows considerable reduction in the size in a few cases after 5 to 14 weeks[29].

RADIOTHERAPY

Radiation therapy for treatment of OSSN is used rarely. Recurrent and incompletely excised invasive OSSN are treated occasionally with contact radiotherapy. Sources such as strontium-90, iodine-125, radium were used earlier. Radiotherapy is useful when tumour shows extensive invasion or if the tumour is not completely excised.

Due to higher incidence of complications (corneoscleral break down at the treatment site, tissue necrosis) and longer duration of treatment required, radiation therapy is rarely used nowadays.

CLINICAL COURSE AND OUTCOMES

Cases of OSSN in which complete surgical excision has been done and confirmed by histopathological examination, are generally declared cured. However, there is a considerable risk of recurrence in cases where the tumour is incompletely excised.[12]

STAGING OF INVASIVE OSSN:

Staging of OSSN is important in prognosis. Higher stage of tumour is associated with worse prognosis.[40]

AJCC STAGING OF CONJUNCTIVAL CARCINOMA PRIMARY TUMOUR(T) STAGE

TX - Primary tumor cannot be assessed

T0 - No evidence of primary tumor

Tis - Carcinoma in situ

T1 - Squamous cell carcinoma 5 mm or less in greatest dimensions T1 stage and beyond represent invasive cancer

T2 - Squamous cell carcinoma >5mm in greatest dimension, without invasion of adjacent structures. (Excludes

carcinomas invading cornea, eye, forniceal conjunctiva, tarsus, lacrimal punctum, canaliculi, plica, caruncle, anterior or posterior eyelid lamella, or eyelid margin)

T3 - Squamous cell carcinoma invades adjacent structures but not orbit (Includes involvement of adjacent structures excluded in T2)

T4 - Squamous cell carcinoma invading orbit with or without further extension

T4a - Squamous cell carcinoma invading bone

T4c - Squamous cell carcinoma invading paranasal sinuses

T4d - Squamous cell carcinoma invading brain

RECURRENCE FOLLOWING SURGERY:

Lee and Hirsthad reported a 17% recurrence rate after excision of conjunctival dysplasia, 40% after excision of Conjunctival Intraepithelial Neoplasias and 30% for Conjunctival Squamous cell carcinomas.[2]

The following are the prognostic factors for recurrence namely,

1) Older age group
2) Large tumours
3) Positive surgical margins
4) Tarsal involvement

5) Tumour invasion
6) Absence of cryotherapy.
7) Increased level of positive expression of proliferation marker Ki-67 in the tumour cells.
8) Absent or inadequate Post- operative adjuvant chemotherapy.

Recurrent cases are treated with combination therapy of surgery with cryotherapy, followed by Chemotherapy with Mitomycin-C.

PROGNOSIS:

The longterm prognosis of OSSN is good. The treatment modalities followed currently are very much effective as the recurrence rate with these procedures is less than 5% and the metastasis to regional lymph nodes is less then 2%.

Aggressive variants of OSSN (mucoepidermoid carcinoma, spindle cell carcinoma) have a bad prognosis[12]

MATERIALS AND METHODS

This is a Retrospective & Prospective study done at the department of Pathology, Regional Institute of Ophthalmology & Government Ophthalmic hospital, Madras Medical College & Government General Hospital, Chennai for a period of 3 years from May2014 to April 2017.

Out of the total 260 ocular tumour specimens during the time period at the department of Pathology, OSSN constituted of about 58 cases (22.31%).

DATA COLLECTION:

This study included all thepatients whose ocular biopsy specimens were histopathologically confirmed as Ocular Surface Squamous Neoplasia (OSSN).

Patients whose complete data could not be obtained and those cases whose original tissue blocks and slides could not be retrieved are excluded from the study. Thus 8 cases were excluded from the study. Benign tumours of the ocular surface stratified squamous epithelium like squamous papilloma, pseudoepitheliomatous hyperplasia, benign hereditary intraepithelial dyskeratosis were also not included in the study.

All the clinical data and findings of the OSSN cases were obtained from the patient files in the pathology registers. The hematoxylin and eosin stained and mounted slides were retrieved from the archives of pathology laboratory.

All the slides were reviewed without the knowledge of previous grading or patient outcome.

The tumour sub typing was done according to the histological pattern seen in the microscopic sections of the tumour. The following histopathological parameters such as dysplasia, loss of polarity, basement membrane integrity, pattern of arrangement of tumour cells, keratinization, nuclear pleomorphism, nucleocytoplasmic ratio, invasion, mitotic figures were analyzed in this study.

Following treatment of OSSN , the appearance of a newly identifiable tumour lesion at the previous site of the tumour is set as a criteria for recurrence .

Ki-67 IMMUNOSTAINING AND INTERPRETATION

Paraffin blocks of 50 cases of OSSN (containing cases from all the grades) were collected for immunohistochemical staining for Ki-67 antigen. The 5 μm full thickness sections were cut from paraffin blocks of the 50 cases and then stained with mouse monoclonal Ki-67 antibody (MIB-1 clone, pre-diluted) purchased from pathnsitu.

The immunohistochemical staining procedure for Ki-67 is given in annexure I.

The slides were then examined with Olympus CX21ilight microscope.The slides are scanned to identify tumour hot spots. Quantitative

analysis was done by ‘real time’ counting Ki-67 positive cells per 500 tumour cells in 10X high power magnification in 3 randomly selected microscopic fields of each slide. The counting was done in real time and then quantified total average number of positive cells stained from each set of 3 randomly analyzed fields and expressed in percentage as Ki-67 proliferation index.[7],[24],[26]

Sections from tonsillar tissue stained for Ki-67 were taken as positive control. Negative controls samples were obtained by avoiding the staining with the primary antibody step during the staining procedure.Ki-67 characteristically shows nuclear positivity. Non-specific staining of the connective tissue or the cytoplasm is considered as negative.

The slides are assessed for the presence and cellular localization of the Ki-67 immunohistochemical staining. If brown nuclear labeling is observed, the staining was considered positive.

STATISTICAL ANALYSIS:

Statistical analysis was carried out using SPSS software version 17. Various tests used in the study were the chi square test for discrete variables, Fisher exact test and the T test for continuous variables.

A significant association between various factors analyzed in the study was found with a level of significance 95% confidence interval and a P cut off value of less than 0.05.

OBSERVATION AND RESULTS

In this study of Ocular Surface Squamous Neoplasia (OSSN), 50histologically confirmed cases during a period of three years were included.

The mean age of occurrence of OSSN in the study was found to be 53.6 years (range: 25 to 84 years). The age distribution of OSSN is given below.

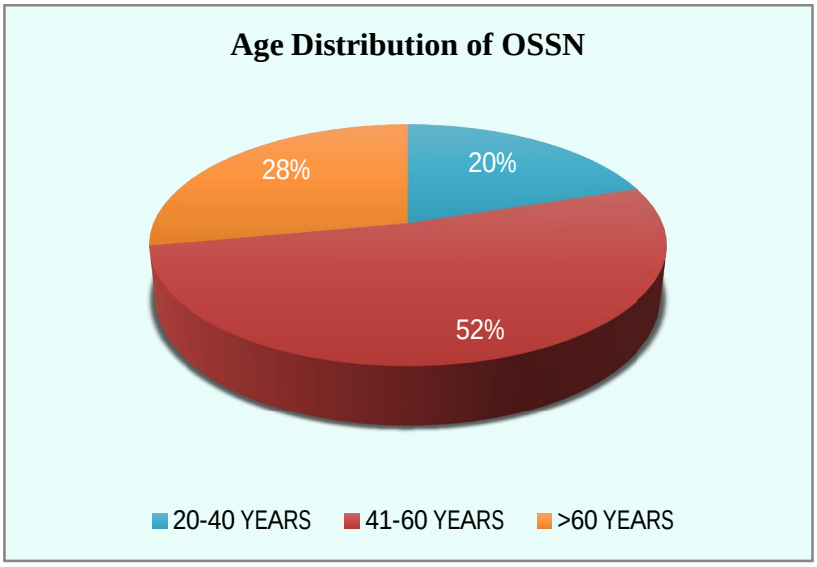
TABLE – 1 : AGE DISTRIBUTION OF OSSN

AGE GROUP	Frequency	Percentage
20-40 YEARS	10	20
41-60 YEARS	26	52
>60 YEARS	14	28
TOTAL	50	100

Mean age: 53.6

Maximum number of cases (52%) were seen in fifth and sixth decades of life taken together. OSSN was least prevalent in less than 40 years of age accounting for about 20% of cases.

CHART-1: AGE DISTRIBUTION OF OSSN



Among OSSN, male patients accounted for 28 cases (56%) and female patients accounted for about 22 cases (44%)in the study, with a male: female ratio of 1.3: 1. There was not a wide variation in the occurrence of OSSN among males and females.

TABLE – 2 : SEX DISTRIBUTION OF OSSN

SEX	Frequency	Percentage
MALE	28	56
FEMALE	22	44
TOTAL	50	100

CHART – 2 : SEX DISTRIBUTION OF OSSN

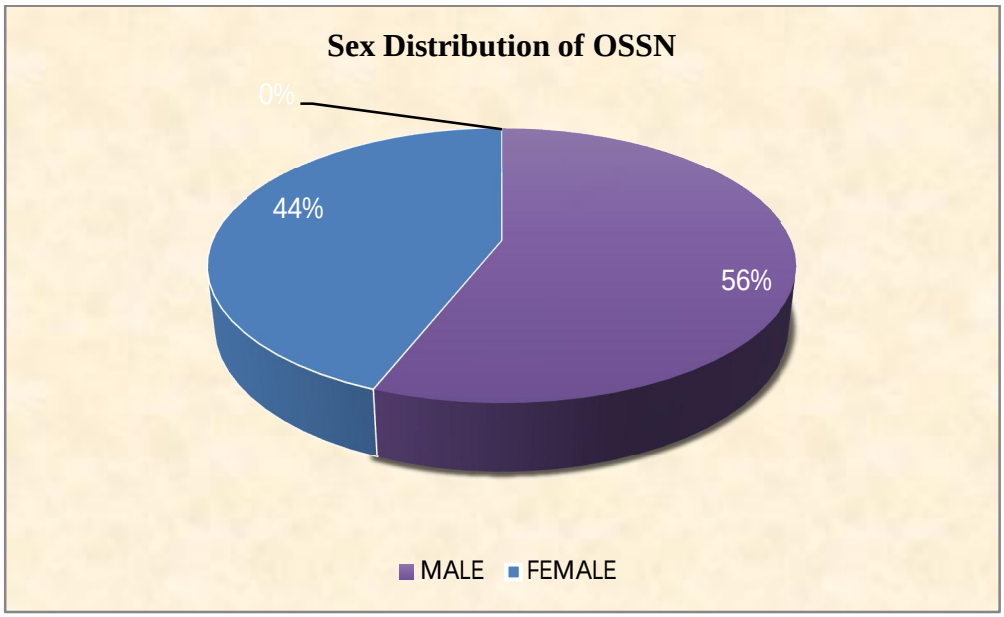


TABLE - 3: DISTRIBUTION OF OSSN WITH RESPECT TO SIDE OF EYE INVOLVED

SIDE	Frequency	Percentage
RIGHT	34	68
LEFT	16	32
TOTAL	50	100

Among OSSN,the right eye was found to have higher numbers, with 34 cases (68%) and the left eye was involved in only 16 cases (32%) in the study.

CHART - 3 : DISTRIBUTION OF OSSN WITH RESPECT TO SIDE OF EYE INVOLVED

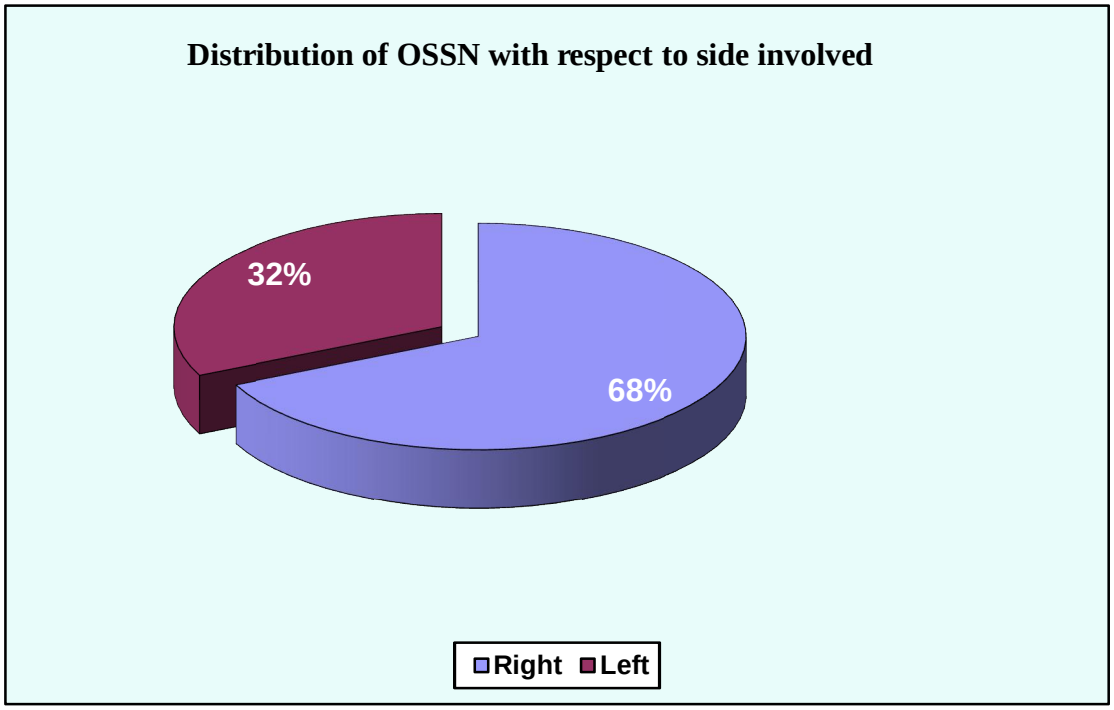
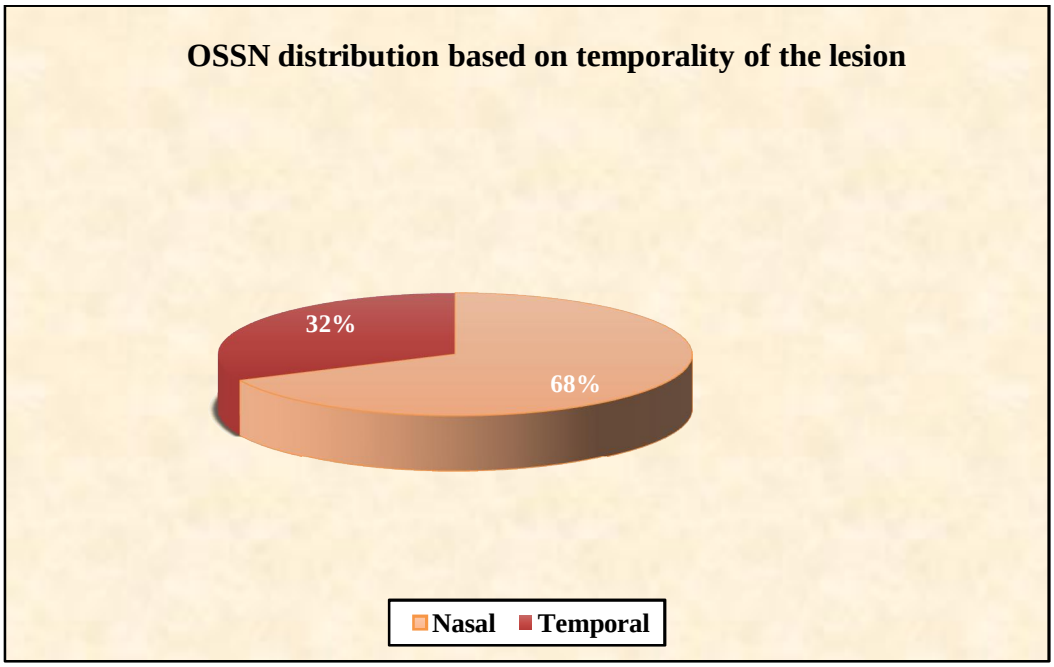


TABLE – 4 : OSSN DISTRIBUTION BASED ON TEMPORALITY OF THE LESION

SITE	Frequency	Percentage
NASAL	34	68
TEMPORAL	16	32
TOTAL	50	100

OSSN was found to be occurring in the nasal quadrant of conjunctiva in higher numbers with 34 cases (68%). The temporal side was found to be involved in only 16 cases (32%) in the study.

CHART – 4 : OSSN DISTRIBUTION BASED ON TEMPORALITY OF THE LESION



All the cases of OSSN in the study were unilateral. The other eye was normal in all the 50 cases.

TABLE – 5 : OSSN DISTRIBUTION BASED ON SITE AND SIDE OF LESION

SIDE	SITE		TOTAL	CHI SQUARE TEST	P VALUE
	NASAL	TEMPORAL			
RIGHT	25	9	34	1.4929	0.22
LEFT	9	7	16		
TOTAL	34	16	50		

Among OSSN, the side of eye involved and temporality of lesion was compared. Right nasal was found to have the highest numbers with 25 cases (50%), followed byrighttemporal with 9 cases (18%), left nasal with 9 cases (18%), and left temporalwhich had least numbers with 7 cases (14%) in the study. Chi square testwas applied had value of 1.4929 and p value of 0.22.

CHART – 5 : OSSN DISTRIBUTION BASED ON SITE & SIDE OF LESION

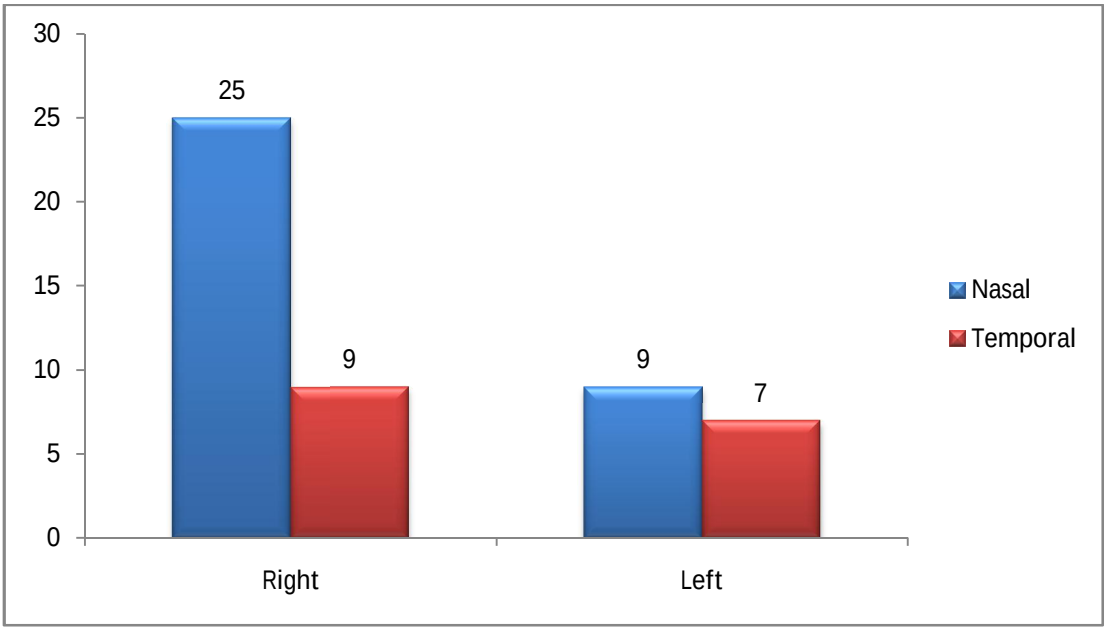


TABLE – 6 : HIV DISTRIBUTION IN OSSN

HIV	Frequency	Percentage
NO	48	96
YES	2	4
TOTAL	50	100

Among OSSN, only 2 cases (4%) were HIV positive and 48 cases (96%) were not infected by HIV. Of the HIV positive cases, one was CIN III and the other was Squamous cell carcinoma, moderately differentiated.

CHART – 6 : HIV DISTRIBUTION IN OSSN

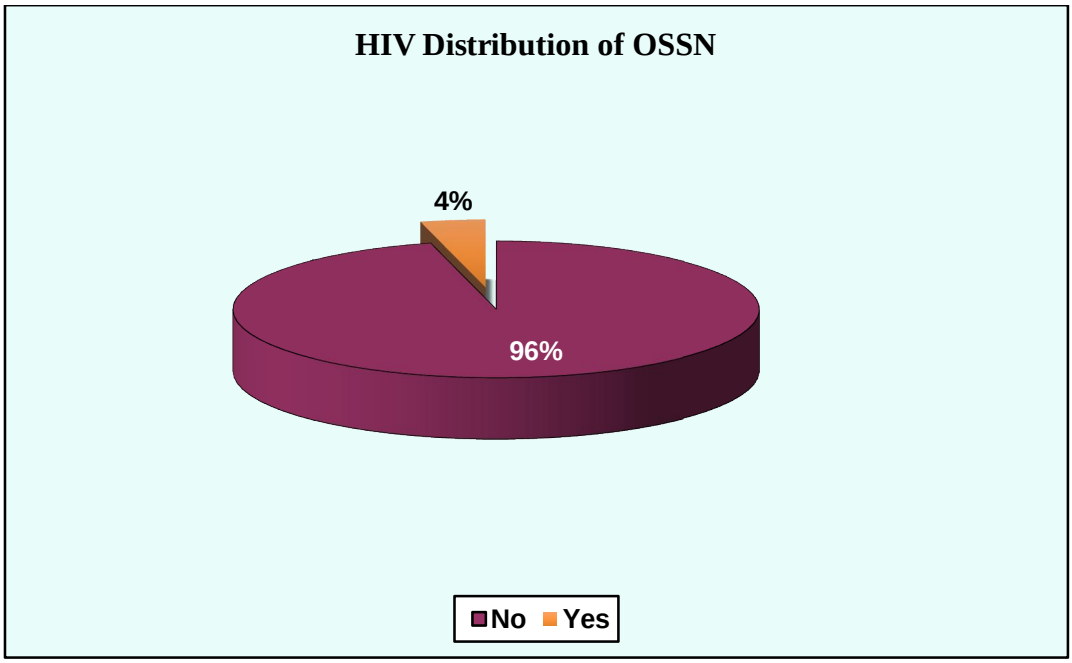


TABLE – 7 : CORNEAL INVOLVEMENT IN OSSN

Corneal involvement	Frequency	Percentage
Present	45	90
Absent	5	10
Total	50	100

Cornea was found to be involved in 45 cases (90%) of OSSN and only 5 cases (10%) of OSSN did not show corneal involvement, in the study.

CHART – 7 : CORNEAL INVOLVEMENT IN OSSN

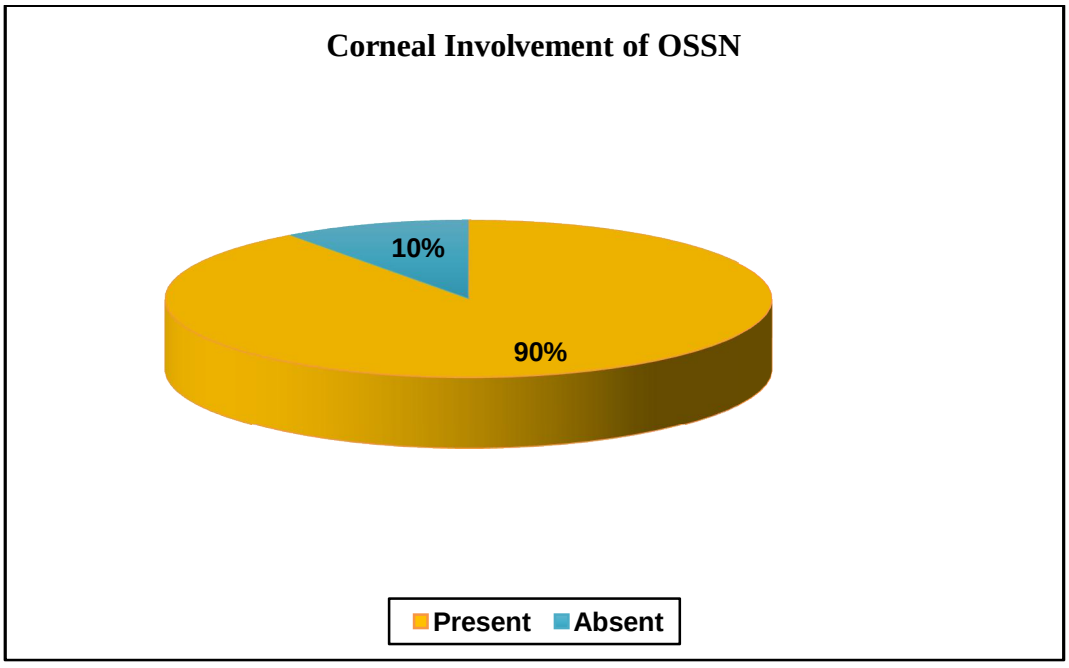


TABLE – 8 : MOST COMMON PRESENTING SYMPTOM OF OSSN

SYMPTOM	Frequency	Percentage
Growth	40	80
Irritation	4	8
Redness	5	10
Vision loss	1	2
Total	50	100

The presenting symptom of OSSN with highest numbers in the study is growth with 40 cases, constituting 80% in the study. Redness of the involved eye is nextcommon symptom with 5 cases (10%), followed by irritation with 4 cases (8%) and vision loss with 1 case (2%).

CHART – 8 : MOST COMMON PRESENTING SYMPTOM OF OSSN

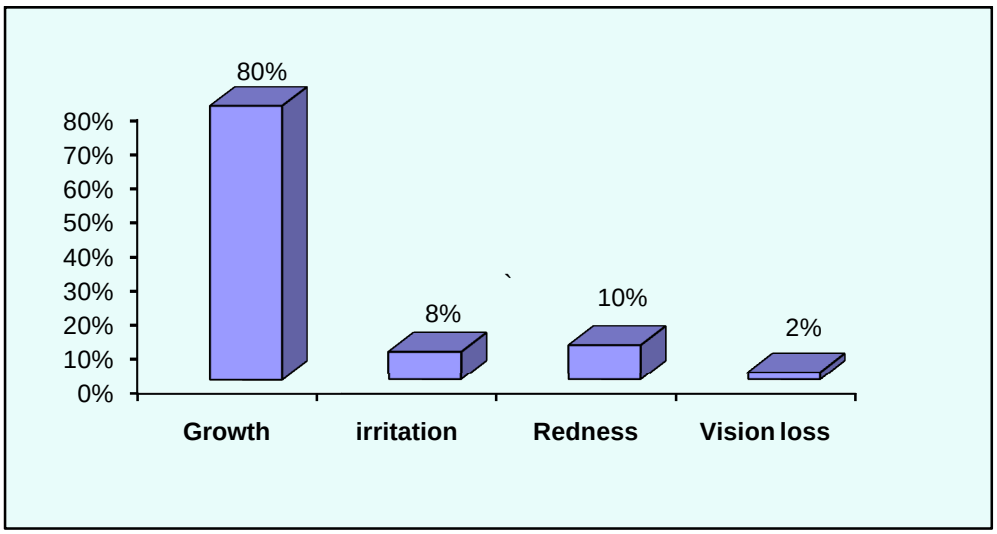


TABLE – 9 : COMPARISION OF PRESENTING SYMPTOM WITH CORNEAL INVOLVEMENT

Corneal involvement	Presenting symptom				p value	Total
	Growth	Irritation	Redness	Vision loss		
Present	38	2	4	1	0.033	45
Absent	2	2	1	0		5
Total	40	4	5	1		50

fisher's exact test

OSSN patients with corneal involvement(total 45 cases) had presented frequent symptom as growth in 38 cases, redness in 4 cases, irritation in 2 cases and lossof vision in 1 case. OSSN patients without corneal involvement (total 5 cases)presented as growth in 2 cases, irritation in 2 cases and redness in 1 case.Fisher's exact test was applied. The p value obtained was 0.033 was significant.

CHART – 9 : COMPARISION OF PRESENTING SYMPTOM WITH CORNEAL INVOLVEMENT

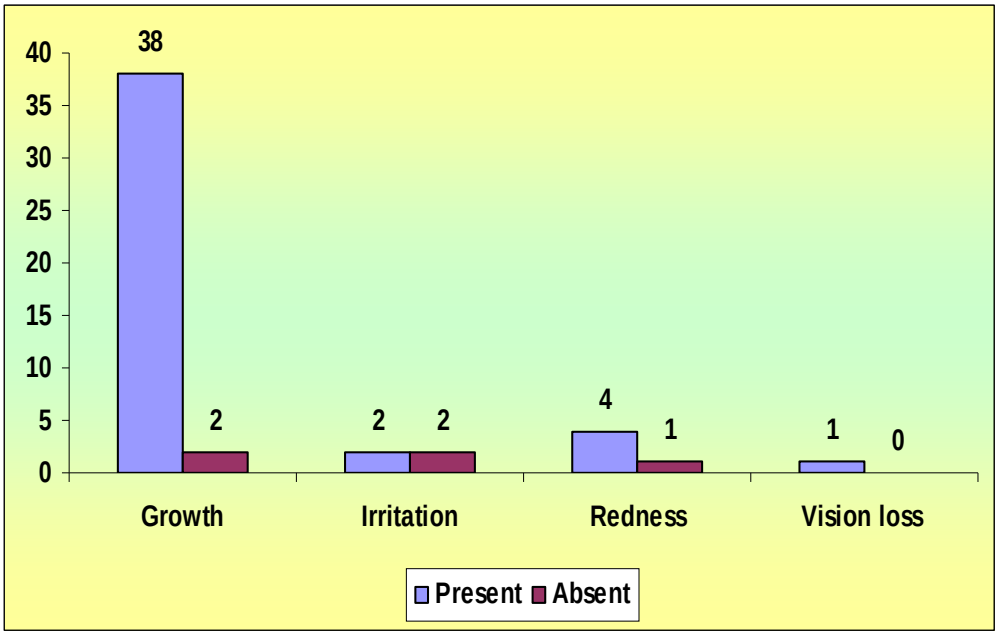


TABLE – 10 : MORPHOLOGICAL TYPES OF OSSN

Type	Frequency	Percentage
Gelatinous	32	64
Leukoplakic	13	26
papillomatous	5	10
Total	50	100

Among OSSN, gelatinous type was found to have more numbers with 32 cases (64%), Leukoplakic type of OSSN was found in 13 cases (26%), papillomatous type having the least number with 5 cases (10%) in the study.

CHART – 10 : MORPHOLOGICAL TYPES OF OSSN

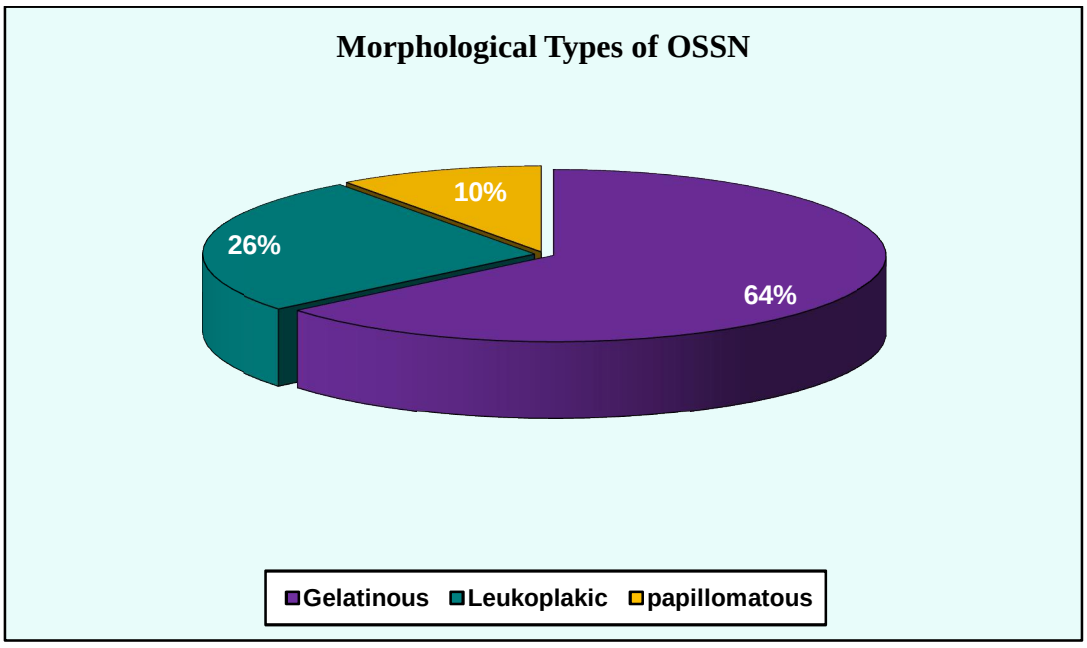


TABLE – 11 : COMPARISION BETWEEN MORPHOLOGICAL TYPE OF LESION AND PRESENTING SYMPTOM

Symptom	Type Of Lesion			P Value	Total
	Gelatinous	Leukoplakic	papillomatous		
GROWTH	26	10	4	0.56	40
IRRITATION	2	1	1		4
REDNESS	4	1	0		5
VISION LOSS	0	1	0		1
TOTAL	32	13	5		50

fisher's exact test

The common presenting symptom in gelatinous type of OSSN was found to beGrowth as seen in 26 out of 32 cases, followed by redness seen in 4 out of 32 cases and irritation seen in 2 cases in the study. Loss of vision was not reported as a presenting symptom in this type.

The common presenting symptom in Leukoplakic type of OSSN was found to be Growth as seen in 10 out of 13 cases. One case each had presented with symptoms of redness, irritation and loss of vision.

The common presenting symptom in Papillomatous type of OSSN was also found to be Growth as seen in 4 out of 5 cases. Irritation was the presenting symptom in 1 case. Redness and loss of vision were not reported as presenting symptoms in this type of OSSN in the study.

Fisher's exact test was applied and p value of 0.56 was obtained.

CHART – 11 : COMPARISION BETWEEN MORPHOLOGICAL TYPE OF LESION AND PRESENTING SYMPTOM

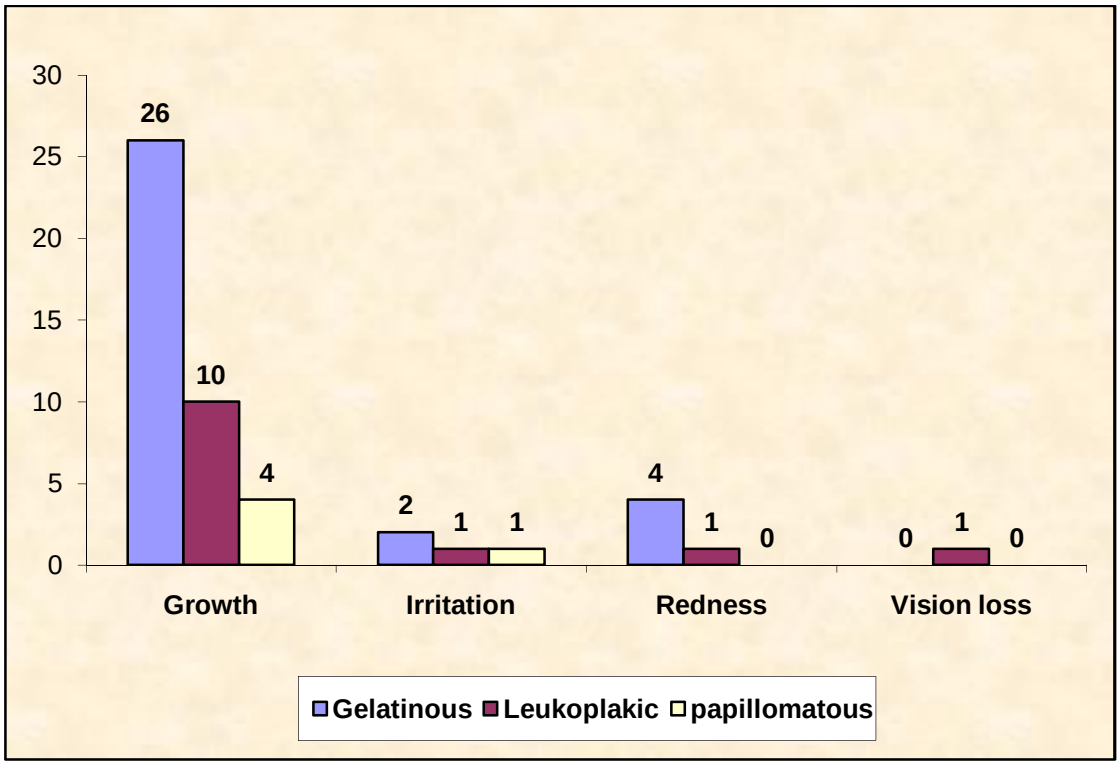


TABLE – 12 : SURGICAL PROCEDURE DONE IN OSSN

SURGERY	FREQUENCY	PERCENTAGE
EXCISON	48	96
EVISCERATION	1	2
EXENTERATION	1	2
TOTAL	50	100

In the study, Excision biopsy was the most commonly performed procedure in 48 cases of OSSN (96%). Evisceration was done in 1 case and Orbital exenteration was done in other.

None of the cases had regional lymph node involvement or distant metastasis.

TABLE – 13 : GROSS SIZE OF OSSN

SIZE (mm)	Frequency	Percentage
0-10	39	78
11-20	9	18
>20	2	4
TOTAL	50	100

The size of lesion as noted by greatest dimension and the cases were grouped into three as less than 10mm, 11-20 mm and more than 20 mm.Among

OSSN, 39 cases (78%) were of size less than 10 mm (all 39 of which were less than 5 mm), 9 cases (18%) were of size between 11-20 mm and 2 cases (4%) wereof size more than 20 mm.

CHART - 12: GROSS SIZE OF OSSN

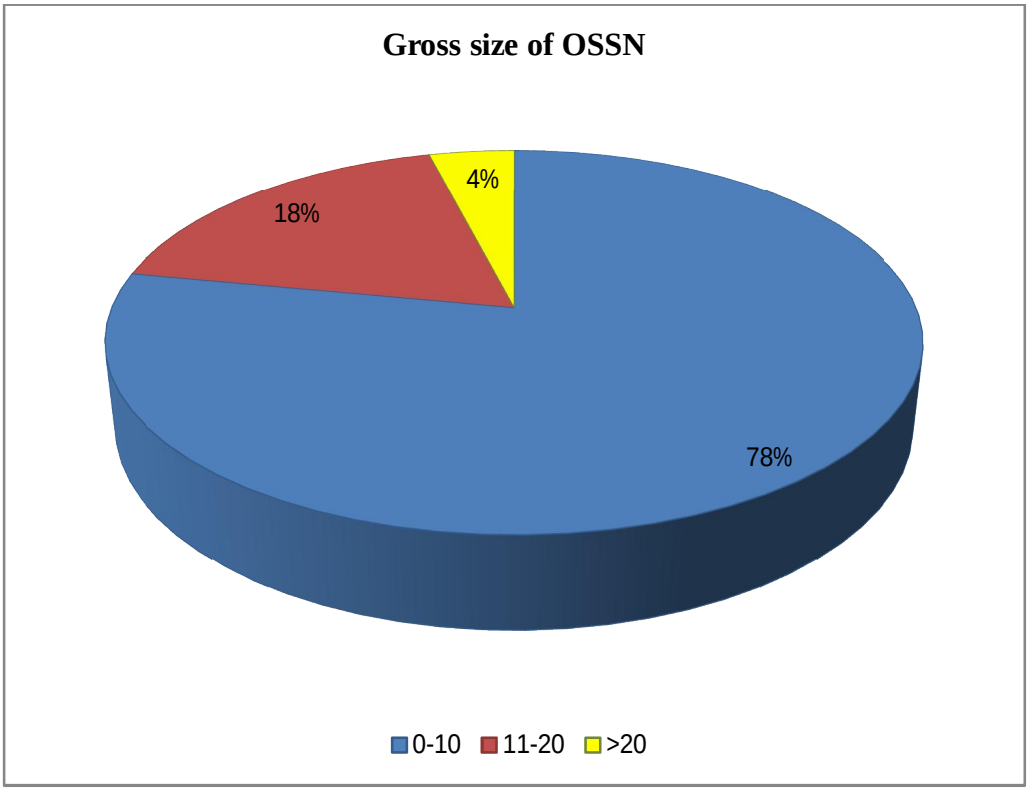


TABLE - 14 : DISTRIBUTION OF DIFFERENT GRADES OF OSSN BASED ON GROSS SIZE

SIZE	TYPE OF OSSN				P value
	CIN I	CIN II	CIN III	SCC	
0-10mm	8	5	25	1	<0.001
11-20mm	0	0	0	9	
>20 mm	0	0	0	2	
Total	8	5	25	13	

fisher exact test

The size of OSSN was found to be higher in Invasive OSSN than in pre invasive OSSN. The mean size of OSSN in the study was 6.3 mm. However, invasive OSSN (SCC) were much larger lesions with mean size of 13.7 and pre-invasive OSSN were smaller in size with a mean of 3.24 mm. Fisher's exact test was applied with p value obtained being <0.001 being statistically significant.

CHART – 13 : DISTRIBUTION OF DIFFERENT GRADES OF OSSN BASED ON GROSS SIZE

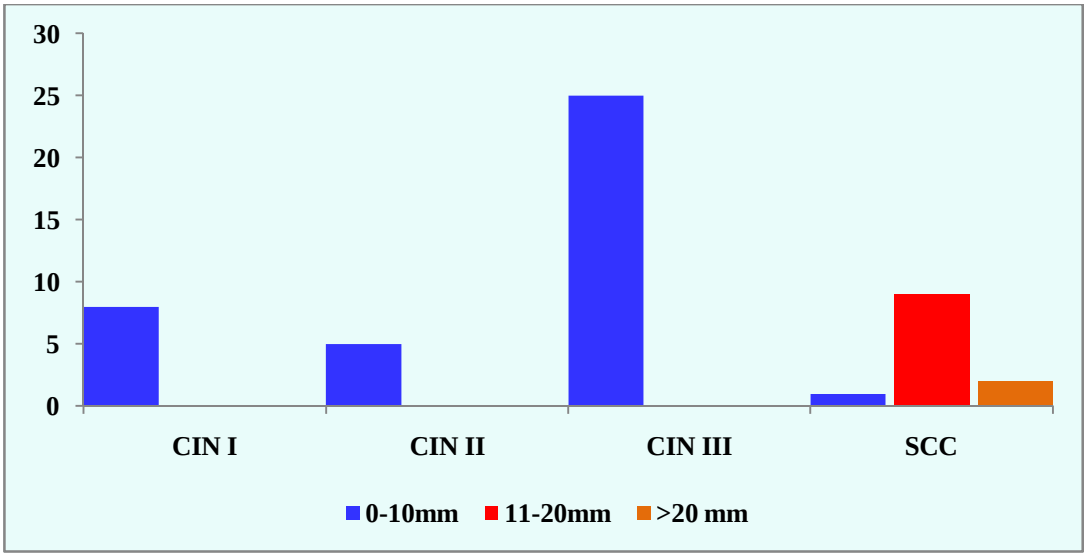


TABLE – 15 : CORRELATION OF IMPRESSION CYTOLOGY AND HISTOPATHOLOGY

Cytology	Frequency	Percentage
Correlated	45	90
Not correlated	5	10
Total	50	100

The impression cytology slides were obtained and diagnosis correlated with the histopathological examination of excised lesions,in 45 cases (90%) of OSSN in the study.Non correlation was seen in 5 cases (10%) ,all of which were pre-invasive OSSN which had been reported as negative for OSSN in cytology .

CHART – 14 : CORRELATION OF IMPRESSION CYTOLOGY AND HISTOPATHOLOGY

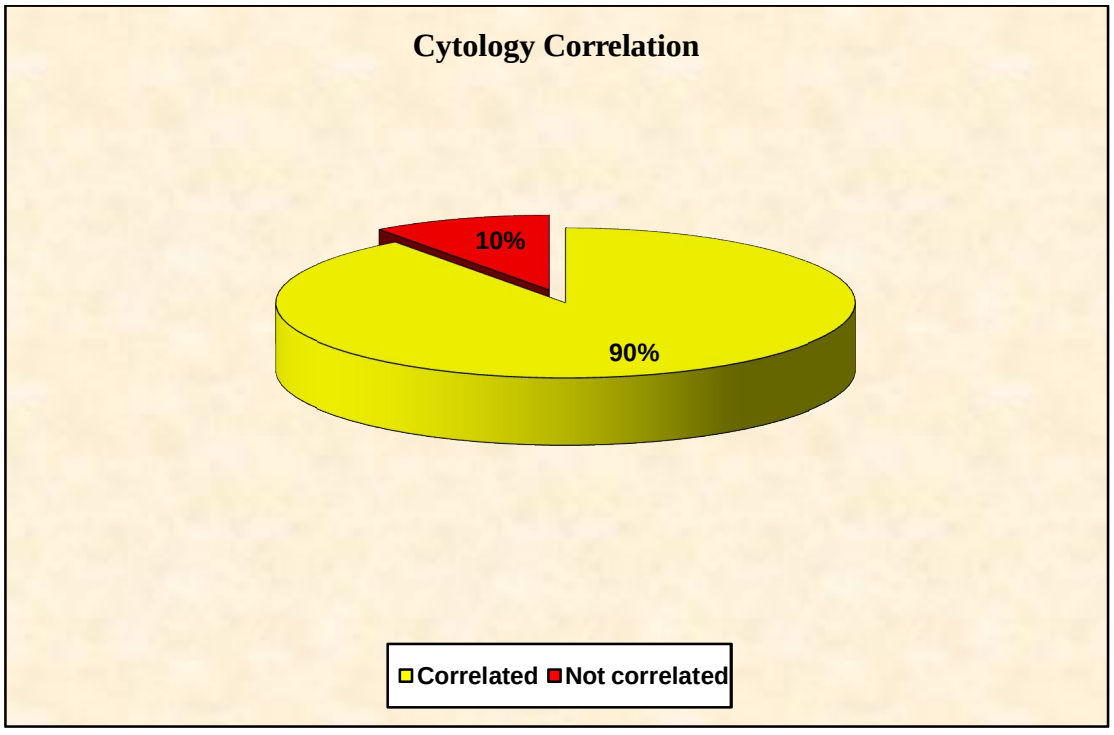


TABLE – 16 : FREQUENCY OF OCCURRENCE OF DIFFERENT HISTOLOGICAL TYPES OF OSSN

Dysplastic Lesion Type	Frequency	Percentage
CIN I	8	16
CIN II	5	10
CIN III/CIS	25	50
SCC	12	24

Among OSSN, pre-invasive OSSN (CIN I,II,III) constituted about 38 cases (76%) and invasive OSSN (all grades of SCC) account for 12 cases(24%) of the cases.

CHART – 15 : FREQUENCY OF OCCURRENCE OF DIFFERENT HISTOLOGICAL TYPES OF OSSN

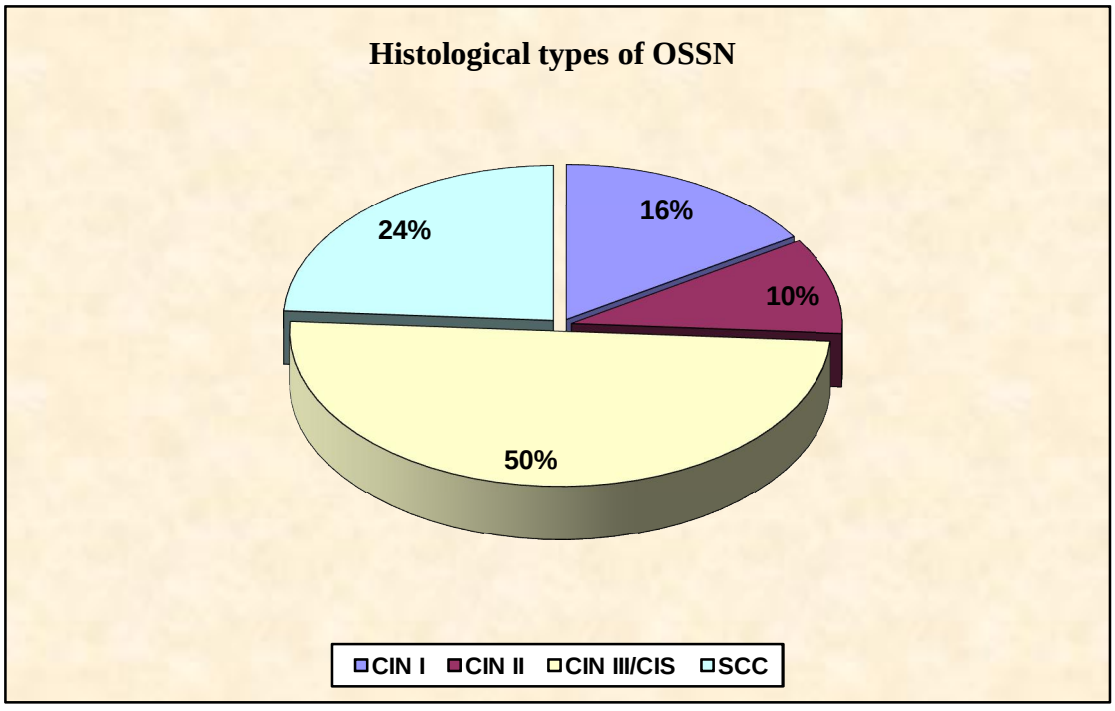


TABLE - 17: COMPARISION OF DIFFERENT GRADES OF OSSN AMONG THE AGE GROUPS

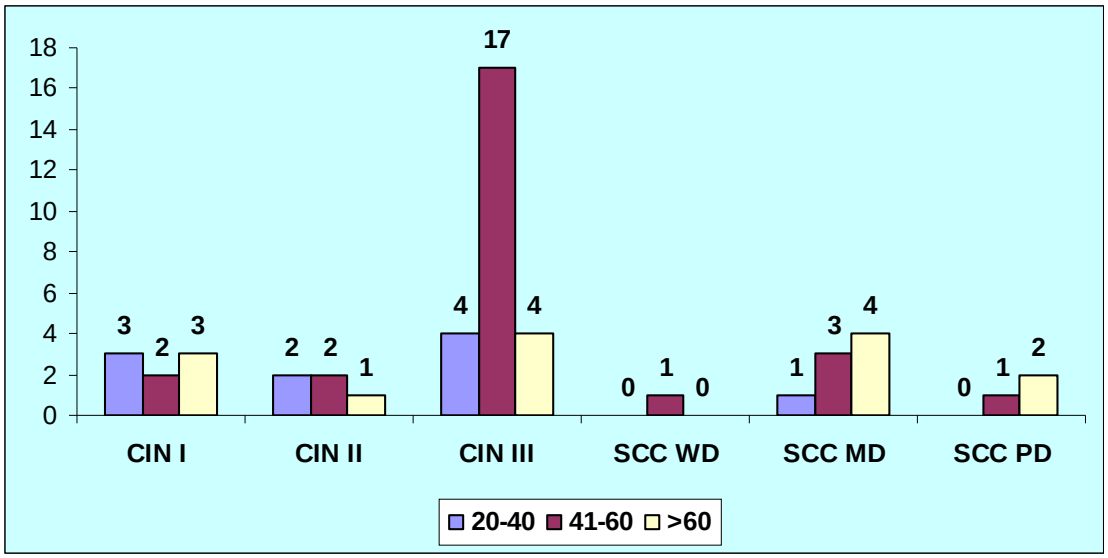
Age group	HPE						p value	Total
	CIN I	CIN II	CIN III	SCC WD	SCC MD	SCC PD		
20-40	3	2	4	0	1	0	0.22	10
41-60	2	2	17	1	3	1		26
>60	3	1	4	0	4	2		14
Total	8	5	25	1	8	3		50

fisher's exact test

Among pre-invasive OSSN, CIN I was distributed without much variation among the three age groups, with a mean age of 50 years. CIN II was also distributed without much variation among the three age groups, with a mean age of 45.8 years. CIN III was seen in much higher numbers in the 41-60 age group with 17 cases, and 4 cases each in 20-40 years and more than 60 year age groups with a mean age of 53.6 years.

Invasive OSSN was more common in the more than 60 yearsage group with 6 cases, followed by 5 cases in the 41-60 year age group, and only 1 case in the 20-40 year age group. Mean age for invasive OSSN is 57.7 years

CHART – 16 : COMPARISION OF DIFFERENT GRADES OF OSSN AMONG THE AGE GROUPS



In the study, 48 out of 50 cases (96%) of OSSN had uninvolved margins on histopathological examination. Only 2 cases (4%) in the study had excised margins showing evidence of tumour infiltration, both being invasive OSSN (one case being moderately differentiated SCC, other poorly differentiated SCC)

TABLE – 18 : INVOLVEMENT OF MARGINS IN OSSN

Margins	Frequency	Percentage
Uninvolved	48	96
Involved	2	4
Total	50	100

CHART - 17: INVOLVEMENT OF MARGINS IN OSSN

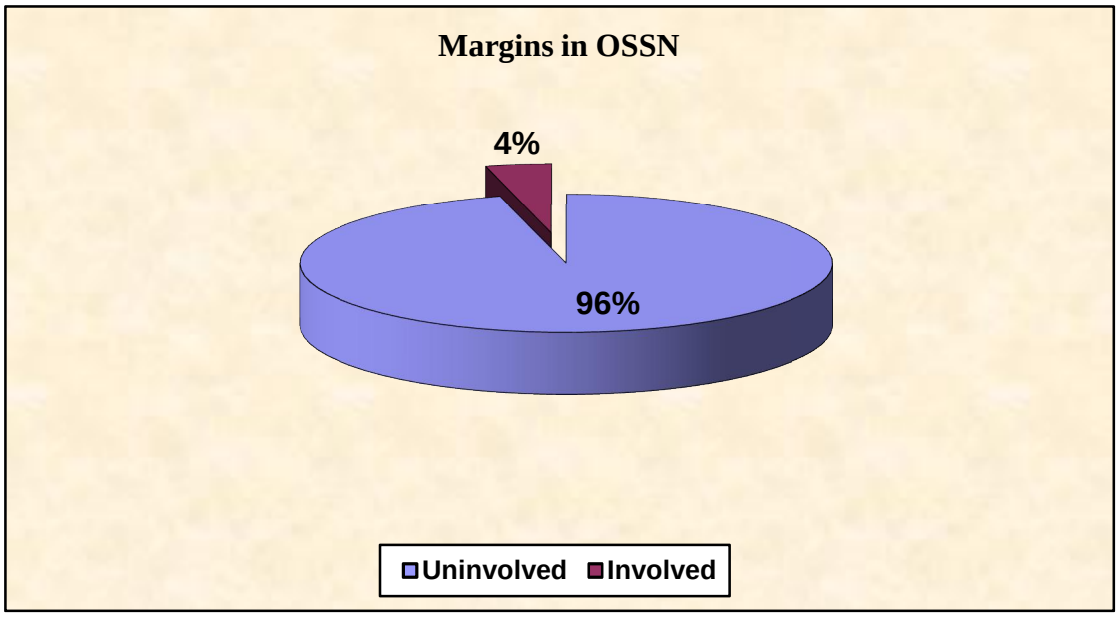


TABLE – 19 : Ki-67 EXPRESSION IN PRE-INVASIVE AND INVASIVE OSSN

Ki67 score	TYPE OF OSSN		Total	P value
	CIN	SCC		
Score I (0-20%)	23	0	23	<0.001
Score II (20-40%)	15	9	24	
Score III (40-60%)	0	3	3	
Total	38	12	50	

fisher exact test

Ki-67 proliferation index as percentage of tumour cells positive was grouped intothree with 0-20% assigned as score I; 21-40% assigned as score II; 40-60% assigned as grade III.

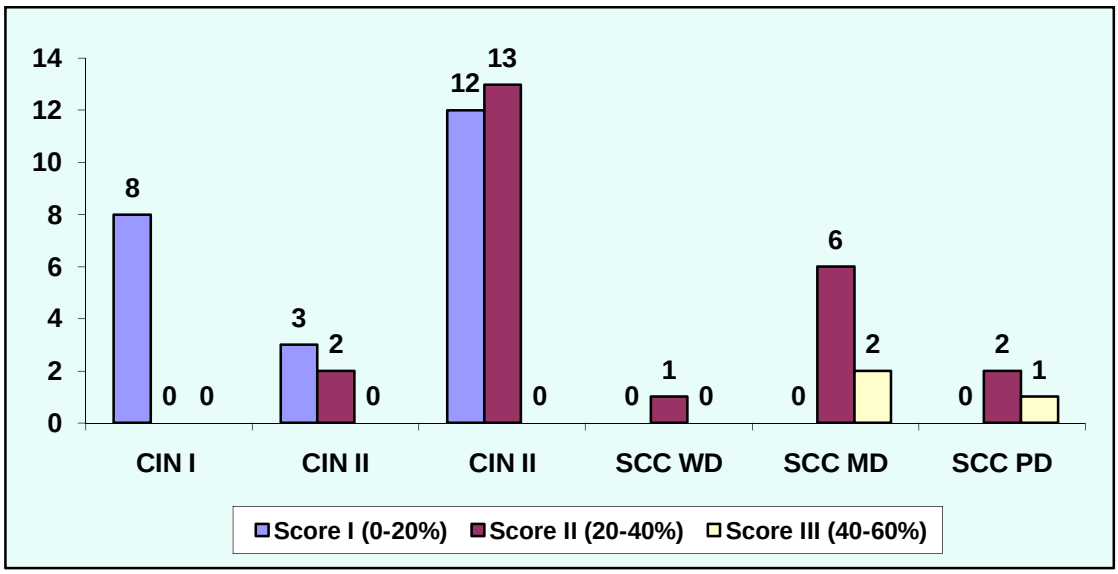
TABLE - 20: KI-67 EXPRESSION IN DIFFERENT GRADES OF OSSN

Ki-67 score	HPE						p value	Total
	CIN I	CIN II	CIN III	SCC WD	SCC MD	SCC PD		
Score I (0-20%)	8	3	12	0	0	0	<0.001	23
Score II (20-40%)	0	2	13	1	6	2		24
Score III (40-60%)	0	0	0	0	2	1		3
Total	8	5	25	1	8	3		50

fisher's exact test

The Ki-67 proliferation index in OSSN ranged between 10 -45% with a mean of 23.48 % in OSSN in the study. Fisher's exact test was applied. p value was obtained as <0.001 implying good statistical significance.

CHART - 18: KI-67 EXPRESSION IN DIFFERENT GRADES OF OSSN



Among pre-invasive OSSN (total 38 cases), CIN I - all 8 cases were in score I ; CIN II - 3 cases had score I and 2 cases had score II; CIN III – 12 cases had score I and 13 cases had score II. Among invasive OSSN (total 12 cases), Ki-67 index in 9 cases had score II and 3 cases had score III.

Invasive OSSN (Squamous cell carcinoma) showed the maximum Ki-67 proliferation index with mean of 36.7%. (range 32-45%). Higher grades of OSSN (poorly differentiated SCC) showing a mean Ki-67 proliferation index of 40.3%, followed by 37.75% in moderately differentiated SCC and 32% in the well differentiated SCC. Pre-invasive OSSN (CIN I,II,III) showed relatively less Ki-67 proliferation index with mean of 18.9%. The p value showed that the association was statistically significant.

TABLE - 21: RECURRENT CASES OF OSSN

Recurrence	Frequency	Percentage
No	48	96
Yes	2	4
Total	50	100

In the study, it was found that only 2 out of 50 cases (4%) of OSSN were recurrent cases. In both cases, the histopathological diagnosis was carcinoma in situ and Ki-67 expression was between 21-40%.

CHART – 19 : RECURRENT CASES OF OSSN

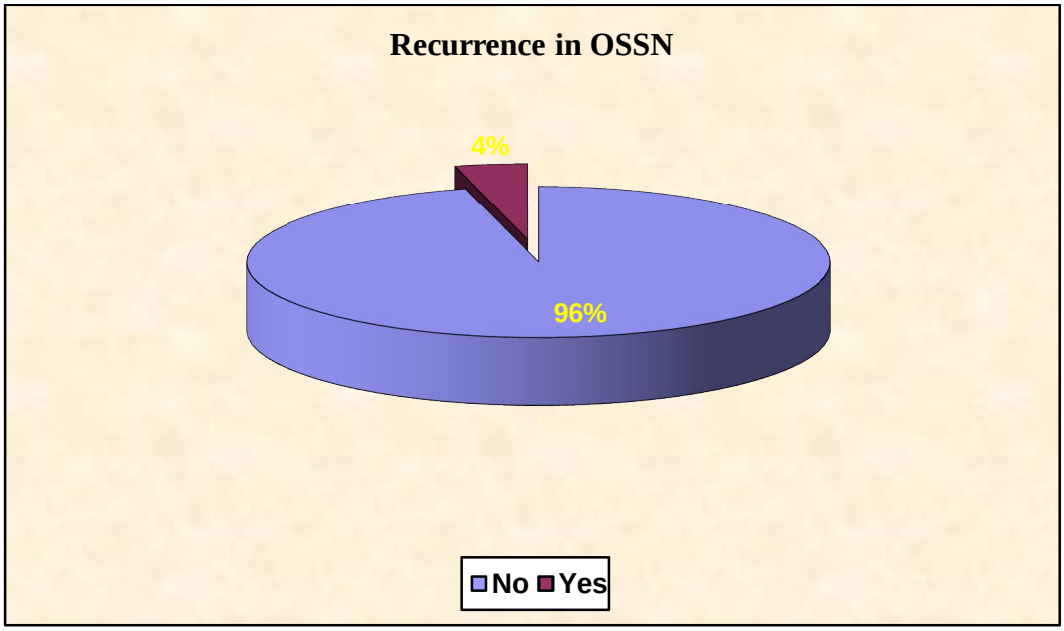


Figure – 1 : HPE 195/14 : CIN GRADE I SHOWING MILD DYSPLASIA

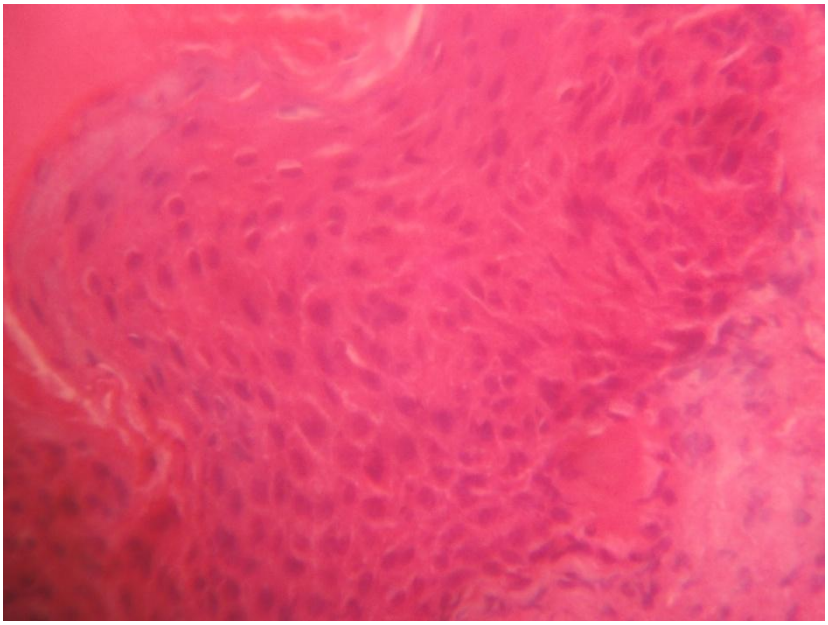


Figure – 2 : Ki-67 IMMUNOSTAINING OF HPE 195/14 : CIN GRADE I

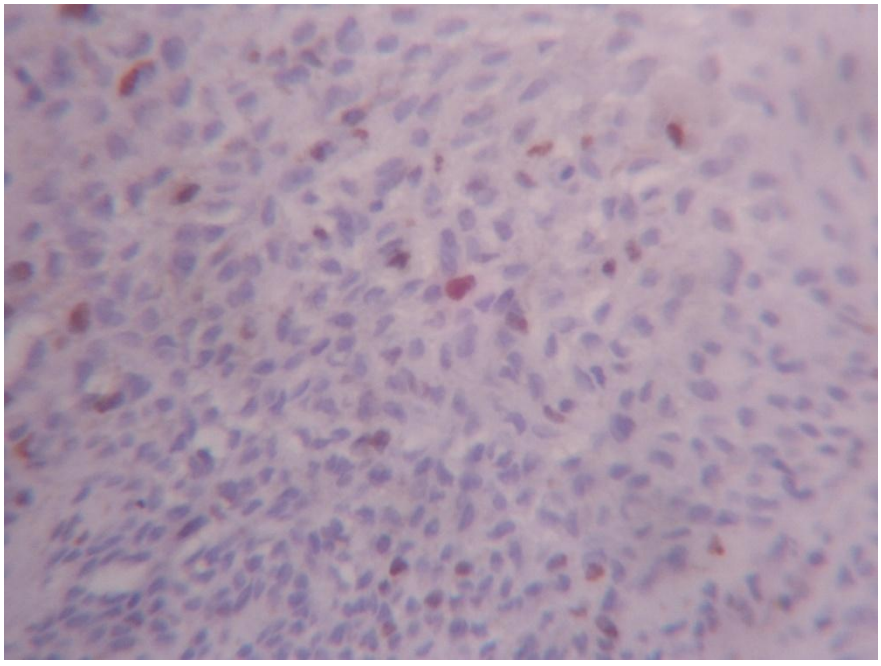


Figure – 3 : HPE 736/15 : CIN II SHOWING MODERATE DYSPLASIA

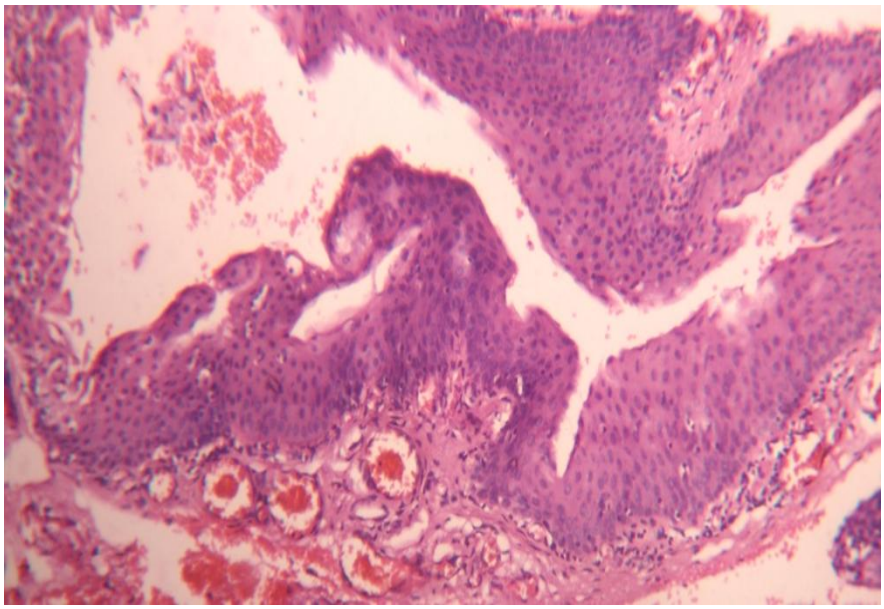


Figure – 4 : Ki-67 IMMUNOSTAINING OF HPE 736/15 : CIN II

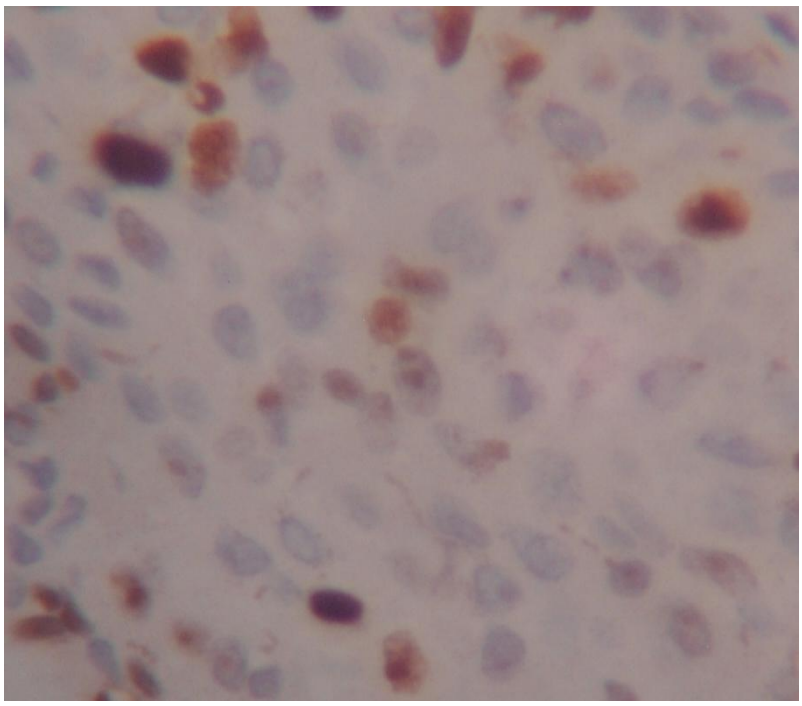


Figure – 5 : HPE 111/14 : CIN III SHOWING FULL THICKNESS SEVERE DYSPLASIA

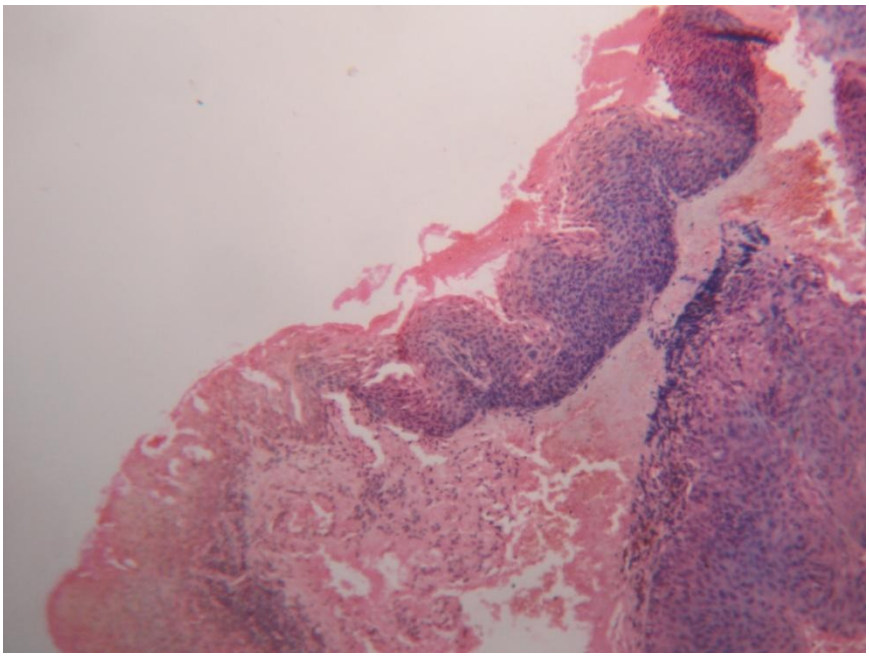


Figure – 6 : Ki-67 IMMUNOSTAINING OF HPE 111/14 : CIN III

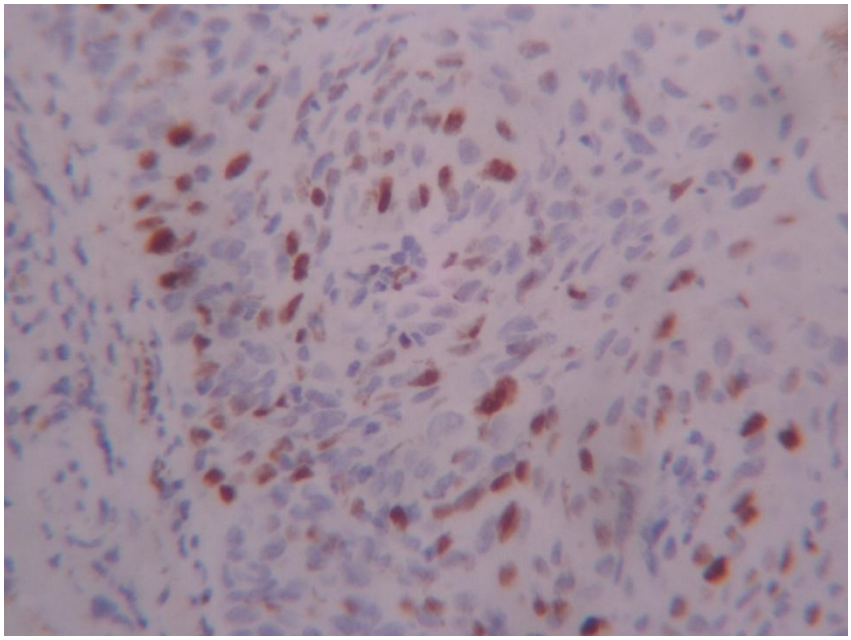


Figure – 7 : HPE 178/16 : SQUAMOUS CELL CARCINOMA , WELL DIFFERENTIATED

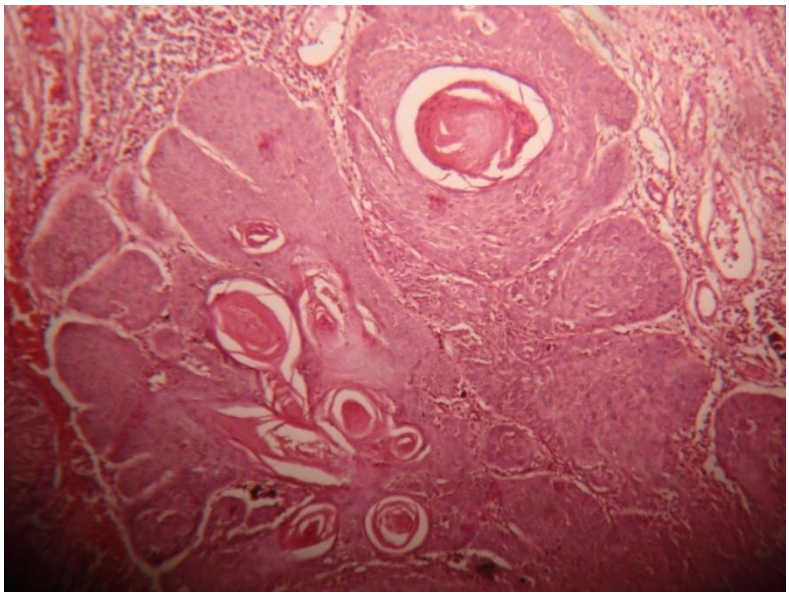


Figure – 8 : Ki-67 IMMUNOSTAINING OF HPE 178/16 :SQUAMOUS CELL CARCINOMA , WELL DIFFERENTIATED

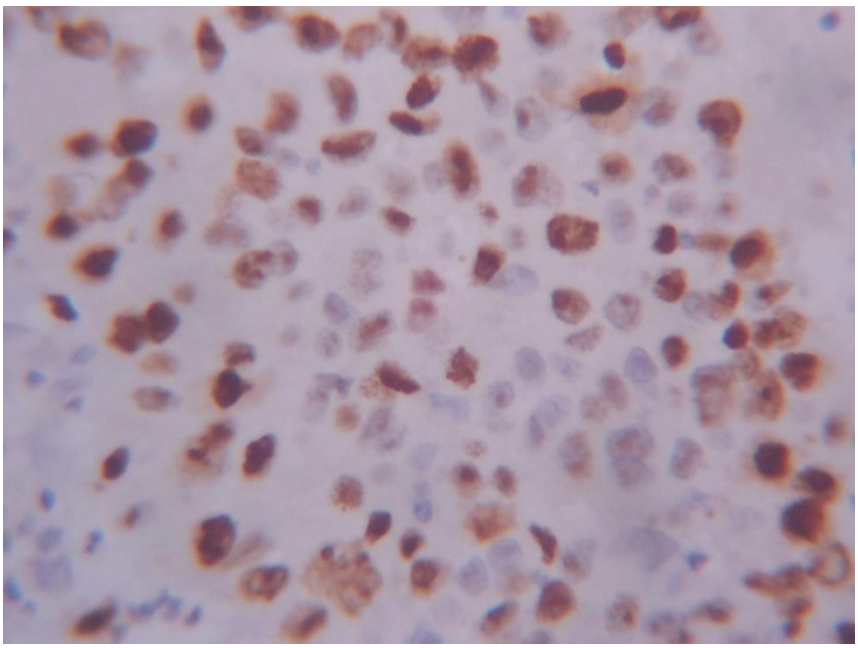


Figure – 9 : HPE 368/15 : SQUAMOUS CELL CARCINOMA, MODERATELY DIFFERENTIATED

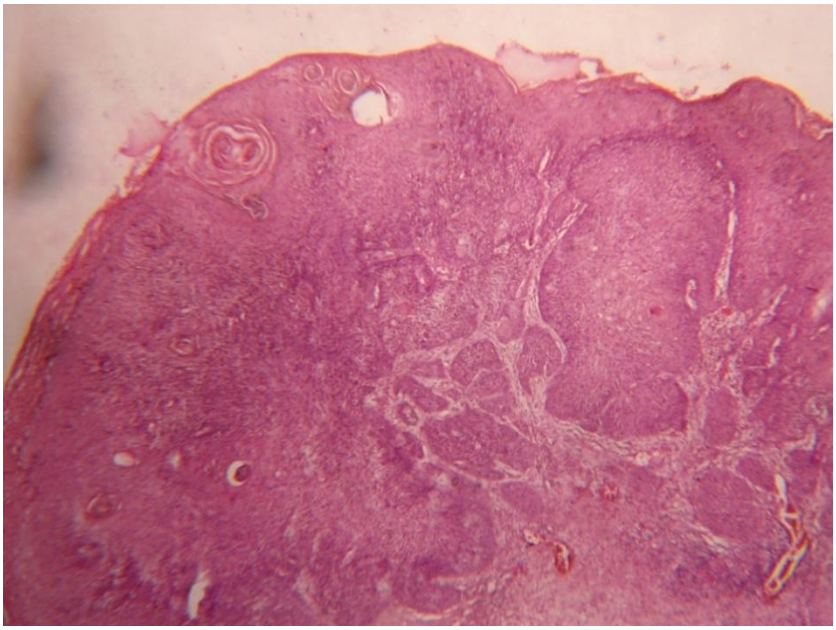


Figure – 10 : Ki-67 IMMUNOSTAINING OF HPE 368/15 : SQUAMOUS CELL CARCINOMA, MODERATELY DIFFERENTIATED

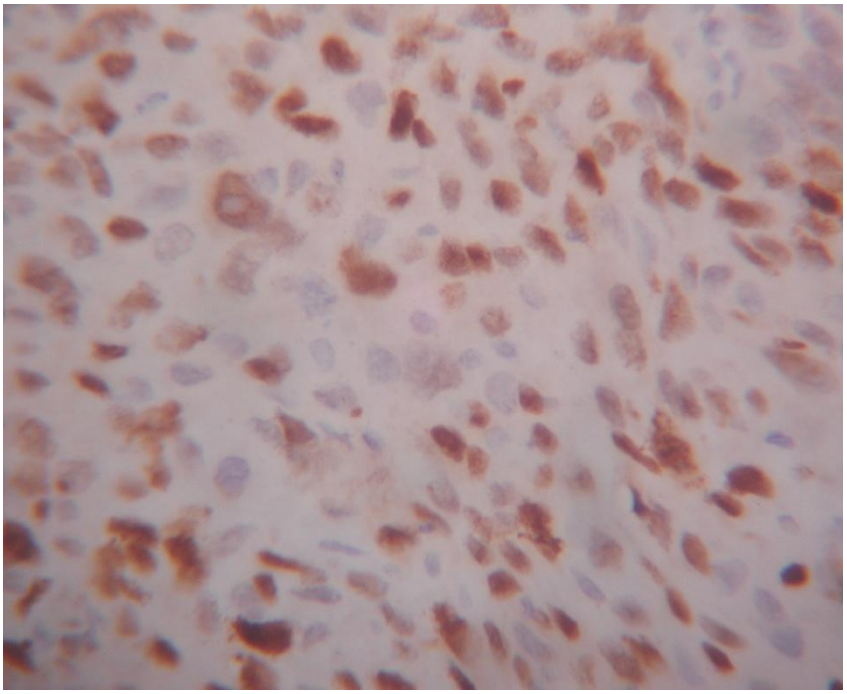


Figure – 11 : HPE 82/15 : SQUAMOUS CELL CARCINOMA, POORLY DIFFERENTIATED

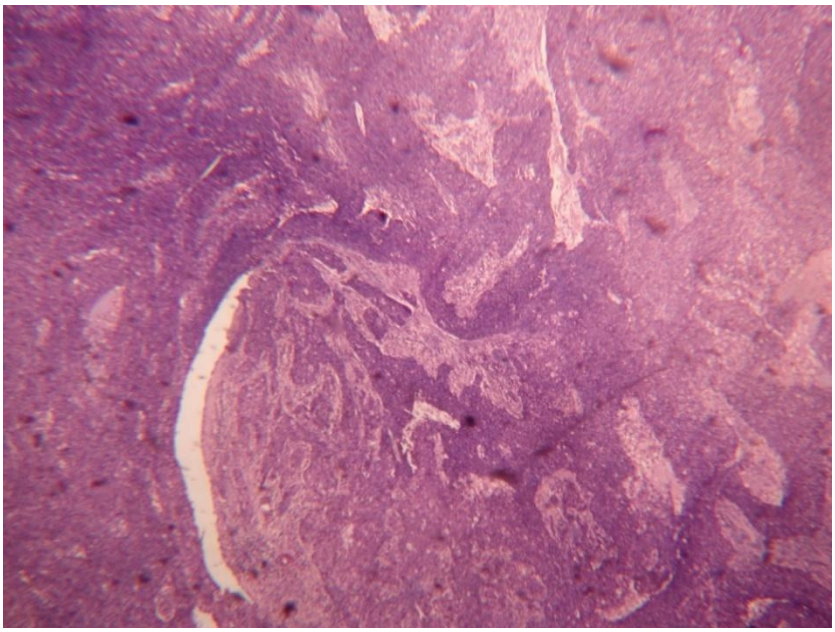


Figure – 12 : Ki-67 IMMUNOSTAINING OFHPE 82/15 : SQUAMOUS CELL CARCINOMA, POORLY DIFFERENTIATED

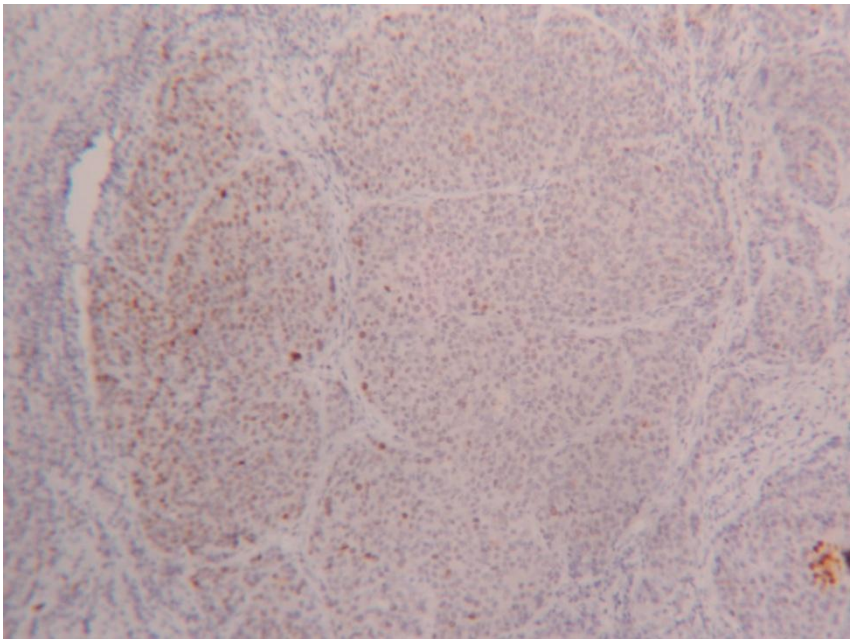


Figure – 13 : CONTROL

KI-67 IMMUNOSTAINING OF TONSIL 10X

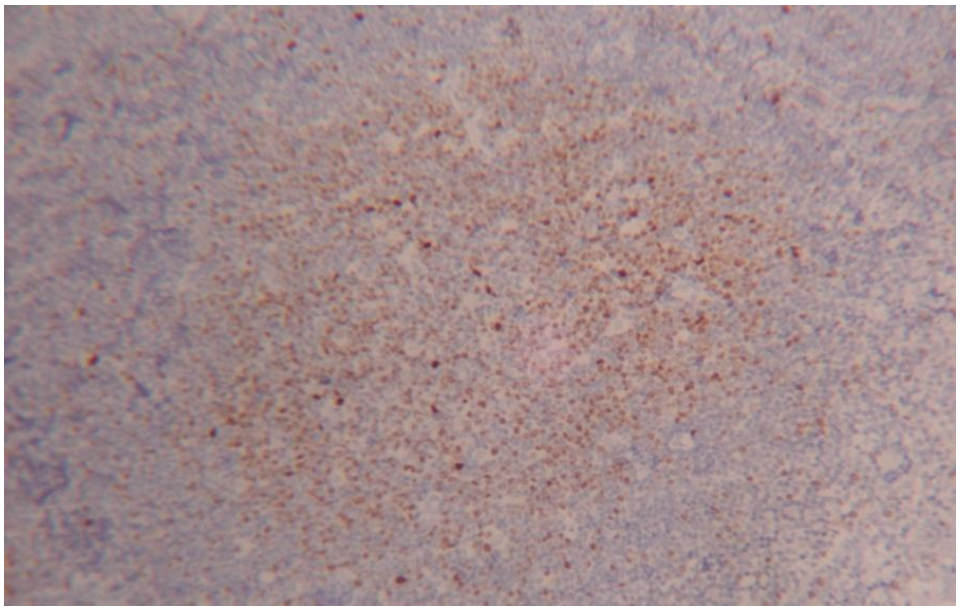
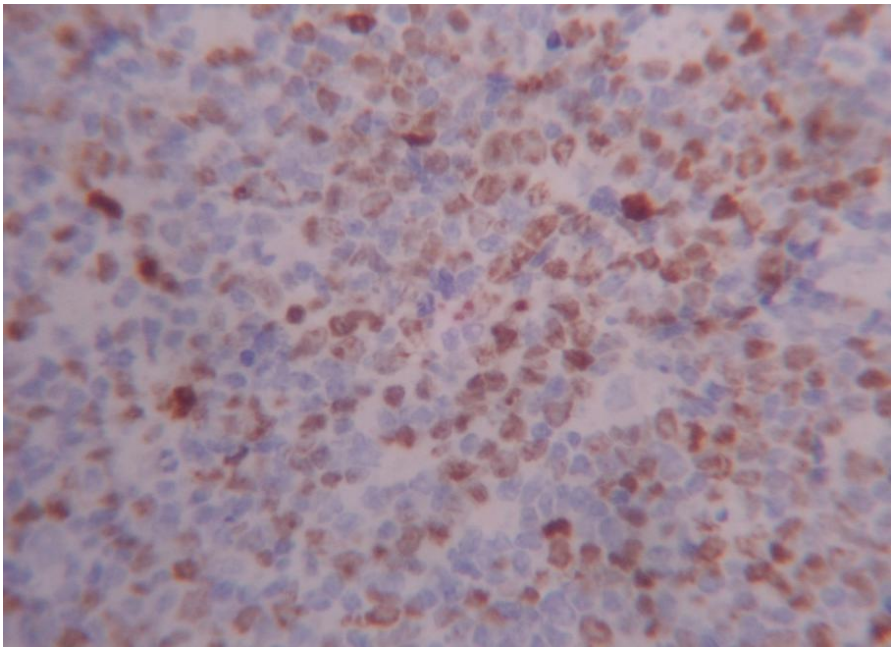


Figure – 14 : Ki-67 IMMUNOSTAINING OF TONSIL 40X



DISCUSSION

Ocular Surface Squamous Neoplasia (OSSN) are the most common ocular surface neoplasms, with varied presentation and comprising of dysplasia, carcinoma in situ and squamous cell carcinoma. OSSN occurs in all races, more commonly in countries that are closer to the equator and in those places where exposure to sunlight is more frequent.

There have been only few studies in southern India, about the clinical and histopathological features of OSSN. This study represents the spectra of presentation in patients with OSSN in our tertiary care center.

In this study, the mean age of the patients presenting with OSSN was 53.6 years (range 25 to 84 years). Though this is similar to many studies like Kim et al[34], Ravindra et al[18], Kavitha et al[33] where older age group of 51 to 60 years is affected commonly, it is lower than the mean age of 45 years reported by Olurin et al[36].

There is not much gender variation of OSSN in this study, only 56 % of the OSSN cases were males, with male to female ratio of 1.3:1. This is in accordance with Ravindra et al[18], where there was not much variation in male:female incidence. However many studies like Erie et al[32], Kavitha et al[33], have shown a higher male preponderance.

Among OSSN, 80 % of the cases in this study presented with growth, similar to the many studies like Erie et al[32] in which 77% presented with growth and Mckelvie et al[5] where 70%cases presented as growth.

Nasal conjunctival tumour location is the most frequent site of invasive OSSN in this study accounting for 66% of the cases of invasive OSSN. This is in accordance with study by Kim et al[34] in which 81% of SCC involved the nasal conjunctiva and cornea. However, study by Kavitha et al[33] had higher incidence of OSSN in temporal location.

All the cases of OSSN in the study presented as unilateral ocular tumour. The other eye was not involved in any of the 50 cases.

HIV infection was found only in 4% of cases of OSSN, in the study. This is very much lower than many studies like Tiong et al[37], Gichuhi et al[35] have reported more than 50% HIV positivity in OSSN cases in African population. However, Ravindra et al[18] also reported only 8.3% of HIV positivity in patients with OSSN similar to this study. Therefore, HIV positivity in OSSN patients may be much lower in India when compared with African nations and western world. However, HIV screening in patients presenting with suspected OSSN is important, as few of these patients have OSSN as a feature of AIDS-related disease.

In this study, among morphological types of OSSN, Gelatinous type of OSSN was the most common type in the study, with 64% of the cases. This is similar to studies like Gichuhi et al where 67% of OSSN presented as

gelatinous mass [35],[12]. However in the study by Kim et al[32] papillomatous type was more common and in study by Ravindra et al[18] Leukoplakic type was more common.

In this study, the impression cytology findings correlated with histopathology findings in 90% of the cases. This is similar to high degree of correlation achieved in studies by Kavitha et al with 76% correlation [33] and Mckelvie et al[22]

The mean size of OSSN in the study was 6.3 mm which is similar to the study by Gichuhi et al[35] in which the mean lesional diameter of OSSN was 6.8 mm. The tumour was larger in size in higher grades of invasive OSSN, similar to study by Mckelvie et al[5].

Surgical excision with wide margin clearance was done in 96% of cases in the study. Evisceration was done in 1 case which had histopatholgical diagnosis as moderately differentiated squamous cell carcinoma and had Ki-67 labeling index of 35%. Orbital exenteration was done in one case which had histopatholgical diagnosis as moderately differentiated squamous cell carcinoma and had Ki-67 labeling index of 41%.Inspite of its slow growing nature, few cases of invasive OSSN may end up in mutilating surgeries like orbital exenteration.

Histopathological diagnosis in the study showed Pre-invasive OSSN (CIN I,CIN II, CIN III) constituting 76% in this study and SCC was only 24%. This was not in accordance with many studies like conducted by Kavitha

et al[33] in which histopathological spectrum showed higher invasive 102 (60%) than non-invasive 68 (40%) lesions. However, this study findings are similar to the study by Andrew A Kao et al study findings which showed preponderance of Carcinoma in situ cases.[38]

The Ki-67 proliferation index in this study had a mean value of 23.48%. Invasive OSSN (Squamous cell carcinoma) showed the maximum Ki-67 proliferation index with mean of 36.7%, with high grade OSSN (poorly differentiated SCC) showing a mean Ki-67 proliferation index of 40.3%, followed by 37.75% in moderately differentiated SCC and 32% in the well differentiated SCC. Pre-invasive OSSN showed relatively less Ki-67 proliferation index with mean of 18.9%. The p value showed that the association was statistically significant. The findings of this study correlates with Ki-67 values seen in other studies like Mckelvie et al[5], Usui et al[9], Chauhan et al[23]. Few studies like Mckelvie et al[5] have reported Ki-67 to be an independent prognostic marker for invasive OSSN.

All the 50 cases presented only with primary tumour. None of the cases had regional lymph node involvement or distant metastasis.

The follow up period of this study was short, and only 4% developed recurrences of OSSN – both were cases of CIN III. This was much lower when compared with studies conducted by Kavitha et al[33] where recurrence was seen in 18.4% of the cases, and Kim et al[34] where recurrence was 37%. Though studies show that recurrence is most commonly associated with

positive surgical margins and higher Ki-67 indices[1][2][5], in this study the 2 cases of recurrent OSSN (both CIN III) had uninvolved surgical margins and had ki-67 proliferation index of 22% and 24%.

The limitation of our study is the limited number of OSSN samples and shorter duration of follow up.

SUMMARY

- Ocular Surface Squamous Neoplasia (OSSN) constituted about 22.31% of the total Ocular tumours during the study period.
- Maximum number cases were encountered in fifth and sixth decades (together 52%)with the mean age of 53.6 years.
- OSSN were almost equally distributed among both sexes, with a male: female ratio of about 1.3 :1. There is not much variation in the sex distribution of OSSN in the study.
- The nasal tumour location in the conjunctiva formed the most common site of occurrence of OSSN constituting 68 %.
- The most frequent presenting system was growth with 80% of cases. 76% of OSSN cases had both corneal involvement and had presented as growth. This was statistically significant.
- The size of OSSN was found to be higher in Invasive OSSN than in pre-invasive OSSN. The mean size of OSSN in the study was 6.3 mm.However, invasive OSSN were much larger lesions with mean size of 13.7 and pre-invasive OSSN were smaller in size with a mean of 3.24 mm.
- Gelatinous type of OSSN was the most common morphological type in the study, with 64% of the cases.

- Pre-invasive OSSN (76%) were more common than invasive OSSN in this study. Among pre-invasive OSSN, CIN III was more common(50%) than CIN I and CIN II.
- Majority of the cases of invasive OSSN (SCC)belonged to moderately differentiated grade, with well differentiated and poorly differentiated SCC accounting for minimum of invasive OSSN cases in the study.
- Immunohistochemical expression of Ki-67 was seen in all cases of OSSN in the study with proliferation index ranging between 10-45% with mean of 23.48%
- Invasive OSSN (Squamous cell carcinoma) showed the maximum Ki-67 proliferation index with mean of 36.7%. (range 32-45%)
- In invasive OSSN (SCC), immunohistochemical expression of Ki-67 was more in high grade OSSN(poorly differentiated SCC) with a mean Ki-67 proliferation index of 40.3%; it decreased to 37.75% in moderately differentiated SCC and 32% in the well differentiated SCC.
- Pre-invasive OSSN showed relatively less Ki-67 proliferation index with mean of 18.9% (range 10-27%)
- The association between the percentage of Ki-67 proliferation index and the grade of the tumour, were significant statistically, with higher grades showing higher Ki-67 index.

CONCLUSION

This study of Ocular Surface Squamous Neoplasia (OSSN) from a tertiary eye care center in southern India is a hospital-based study and may not represent the true incidence of disease in the community.

Many patients presented in the old age with a mean age of incidence of 53.6 years. There was not much variation in sex distribution. Since most of patients present with minimal symptoms in the early stage, ophthalmologists should have high degree of alertness, in order to recognize, evaluate and histopathologically confirm as OSSN. The newer diagnostic techniques like impression cytology, help the clinician in diagnosis, treatment and follow up of OSSN.

Histopathology is the gold standard in diagnosing the distinct types and grades of OSSN. Also it gives a good insight about the clinical outcome of the disease. Pre-invasive OSSN was more common in this study than invasive OSSN.

In the study, Ki-67 proliferation index had shown a statistically significant association with the grade of OSSN, with high grade invasive OSSN showing higher Ki-67 proliferation index and pre-invasive lesions showing a lower Ki-67 index. Enhanced Ki-67 expression may contribute to the aggressive nature of OSSN. Larger sample size and follow up for more years could throw more light on the progression of OSSN and added value of this marker in OSSN.

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ANNEXURE I

THE IMMUNOHISTOCHEMICAL STAINING PROCEDURE FOR KI-67

1. Formalin fixed paraffin embedded blocks were used to get sections of 4 μ m thickness. These sections are coated in gelatin chrome alum pretreated slides.
2. These glass slides must be incubated overnight at 58 degrees Celsius.
3. Deparaffinisation is done using two changes of xylene one each lasting for 15 minutes.
4. Dehydration of tissues are done with two changes of absolute alcohol each lasting for 5 minutes.
5. The sections are then placed in two changes of distilled water each lasting for 5 minutes.
6. Process of antigen retrieval:- Preheat the freshly prepared TRIS antigen retrieval buffer for 4 minutes at 800 watts in microwave oven. This is followed by heating the sections in TRIS buffer for 20 minutes as follows:- (i) 800 watts for 5 minutes (ii) 640 watts for 10 minutes and (iii) 480 watts for 5 minutes.
7. Cool the slides to room temperature.
8. Wash the slides in running tap water for 5 minutes and distilled water for 5 minutes.
9. Now wash the slides in phosphate buffer for 5 minutes.
10. Treat the slides with hydrogen peroxide for 5 minutes.
11. Wash the slides in old and new phosphate buffer for 2 and 5 minutes respectively.
12. The primary antibody (MIB-1) is now added and is made to react for a period of 30 minutes.
13. Wash the slides with phosphate buffer and treat the slides with poly excel target binder for 12 minutes.

14. Wash the slides with phosphate buffer and add horse radish peroxidase and allow it react for 12 minutes.
15. Wash the slides with phosphate buffer and then add DAB chromogen (prepared by adding 1ml of DAB buffer and 1 drop of DAB chromogen)
16. The sections are counter stained with hematoxylin 30 seconds, washed for 5 minutes in running tap water, air dried, cleared in xylene and mounted.

INFORMATION SHEET

- We are conducting a study on Ocular Surface Squamous Neoplasia (OSSN) among patients attending Regional Institute of Ophthalmology and Government Ophthalmic Hospital, Madras Medical College, Chennai and for that your specimen may be valuable to us.
- The purpose of this study is to analyse Clinicopathological profile of Ocular Surface Squamous Neoplasia (OSSN) and do special tests (Ki-67 expression) in cases of Ocular Surface Squamous Neoplasia (OSSN)
- We are selecting Ocular Surface Squamous Neoplasia (OSSN) cases and if your specimen is needed, we may be using your specimen to perform tests which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator

Signature of participant

Date:

INFORMED CONSENT FORM

Title of the study : **" A study of Clinicopathological features and Proliferation marker Ki-67 expression in Ocular Surface Squamous Neoplasia "**

Name of the Participant :
Name of the Principal (Co-Investigator) :
Name of the Institution : Madras Medical College
Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in **"A study of Clinicopathological features and Proliferation marker Ki-67 expression in Ocular Surface Squamous Neoplasia"**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the ocular tumors were subjected to histopathological examination
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me. I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

ஆராய்ச்சி தகவல்தாள்

ஆராய்ச்சி தலைப்பு

விழியின் மேற்பரப்பில் செதிள் மிகைப்புடன் பற்றி மருத்துவ நோயியல் சார்ந்த மற்றும் Ki-67 எனும் சிறப்பு குறியீடு இட்டு செய்யும் ஆய்வு

ஆய்வாளர் : கோகுலகண்ணன்.R
நோய்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி. சென்னை-3.

தங்களது கண்ணுக்குரிய கட்டிகளின் சதைப் பரிசோதனைக்கு பயன்பட்ட மெழுகுக் கட்டிகளை வைத்து ஆராய்ச்சி செய்யப்படுகிறது.

அரசினர் கண் மருத்துவமனை, சென்னை மருத்துவக் கல்லூரிக்கு வரும் நோயாளிகளின் விழியின் மேற்பரப்பில் செதிள் மிகைப்புடன் எனும் கட்டிகளை பற்றிய ஆராய்ச்சி செய்யப்படுகிறது.

விழியின் மேற்பரப்பில் செதிள் மிகைப்புடன் எனும் கட்டியின் மருத்துவ நோயியல் சார்ந்த விவரங்களையும் Ki-67 எனும் சிறப்பு குறியீடு இட்டு அதன் பயனை மதிப்பீடு செய்வதும் எனது ஆய்வின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் பரிசோதனைக்கு உட்படுத்தப்பட்ட தங்களின் திசுக்களையும் அதன் தகவல்களையும் மற்றும் வெறப்பட்ட திசுக்களை எடுத்து சிறப்பு பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களையும் ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ எந்தவித பாதிப்பும் ஏறபடாது என்பதை தெரிவித்துக்கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆய்வின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக்கொள்கிறேம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில்தான் இருக்கிறது. மேலும் நீங்கள் எந்த நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின் வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த பரிசோதனையின் முடிவுகளை ஆராய்ச்சியின்போது அல்லது ஆராய்சியின் முடிவில் தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த ஆய்வை பற்றிய சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர் மரு.கோகுலகண்ணன்.R, செல்: 9629928599

பங்கேற்பவரின் கையொப்பம் ..... இடம்..... தேதி.....
கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம்..... தேதி.....

ஆய்வாளரின் பெயர் .....

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு

விழியின மேற்பரப்பில் செதிள் மிகைப்புடன் பற்றி மருத்துவ நோயியல் சார்ந்த மற்றும் Ki-67 எனும் சிறப்பு குறியீடு இட்டு செய்யும் ஆய்வு

சென்னை மருத்துவக் கல்லூரி நோய்க்குறியியல் துறையில் பயிலும் முதுகலை மருத்துவர் கோகுலகண்ணன். R. அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழு மனதுடன் சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விவரங்களை புரிந்துகொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்துகொண்டேன்.

நான் விழியின் மேற்பரப்பில் செதிள் மிகைப்புடன் குறித்த இந்த ஆராய்ச்சியின் விவரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக்கொண்டேன்.

நான் என்னுடைய சய நினைவுடன் மற்றும் முழு சதந்திரத்துடன் இந்த மருததுவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்.

எனக்கு அறுவை சிகிச்சை செய்யப்பட்டு நோய்குறியியல் துறையில் சதைப் பரிசோதனைக்கு பயன்பட்ட மெழுகு கட்டிகளை வைத்து ஆராய்ச்சி மற்றும் சிறப்புப் பரிசோதனைகளை செய்துகொள்ள சம்மதம் தெரிவிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் ..... இடம்..... தேதி.....

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம்..... தேதி.....

KEY TO MASTER CHART

M	-	Male
F	-	Female
R	-	Right
L	-	Left
N	-	Nasal
T	-	Temporal
HIV+	-	Human Immunodeficiency virus positivity
Cornea inv	-	Corneal involvement
Y	-	Yes
N	-	No
GEL	-	Gelatinous
LEUK	-	Leukoplakic
PAP	-	Papillomatous
C	-	Correlated
NC	-	Non Correlated
Exc Bx	-	Excision Biopsy
Evisc	-	Evisceration
Exentr	-	Exenteration
I	-	Involved
UI	-	Uninvolved
HPE	-	HistoPathological Examination
CIN I	-	Conjunctival Intraepithelial Neoplasia grade I
CIN II	-	Conjunctival Intraepithelial Neoplasia grade II
CIN III	-	Conjunctival Intraepithelial Neoplasia grade III
SCC WD	-	Squamous Cell Carcinoma Well Differentiated
SCC MD	-	Squamous Cell Carcinoma Moderately Differentiated
SCC PD	-	Squamous Cell Carcinoma Poorly Differentiated
METS	-	METASTASIS

S.No	HPE No	Age	Sex	Side	Site	Size mm	HIV +	Cornea inv	Presenting complaint	Other eye	Morphology-ical Type	Cytology correlation	Surgical procedure	Margins	HPE Diagnosis	Ki-67 index(%)	Node	Mets	Recur-rence
1	16/14	33	M	R	N	2	N	N	irritation	Normal	GEL	C	Exc bx	UI	CIN I	15	N	N	N
2	43/14	55	M	R	N	4	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	20	N	N	N
3	69/14	33	M	L	N	3	N	N	redness	Normal	GEL	C	Exc bx	UI	CIN II	15	N	N	N
4	72/14	84	F	R	T	4	N	Y	growth	Normal	LEUK	C	Exc bx	UI	CIN III	22	N	N	N
5	82/14	59	M	R	N	2	N	Y	irritation	Normal	PAP	C	Exc bx	UI	CIN III	23	N	N	N
6	100/14	60	F	L	N	11	N	Y	vision loss	Normal	LEUK	C	Exc bx	UI	SCC MD	37	N	N	N
7	111/14	60	F	L	T	5	Y	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	19	N	N	N
8	129/14	25	M	R	N	2	N	Y	redness	Normal	LEUK	NC	Exc bx	UI	CIN I	10	N	N	N
9	163/14	55	F	R	N	6	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	17	N	N	N
10	195/14	42	F	L	N	2	N	Y	growth	Normal	GEL	NC	Exc bx	UI	CIN I	12	N	N	N
11	198/14	65	M	R	T	4	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN I	18	N	N	N
12	215/14	48	F	R	N	5	N	Y	growth	Normal	LEUK	C	Exc bx	UI	SCC MD	33	N	N	N
13	233/14	80	F	R	T	3	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN I	18	N	N	N
14	265/14	60	F	L	N	5	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	21	N	N	N
15	292/14	55	F	L	N	4	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	13	N	N	N
16	.11/15	57	F	R	N	19	N	Y	growth	Normal	PAP	C	Evisc	UI	SCC MD	35	N	N	N
17	49/15	52	M	R	N	4	N	Y	growth	Normal	GEL	NC	Exc bx	UI	CIN II	21	N	N	N

S.No	HPE No	Age	Sex	Side	Site	Size mm	HIV +	Cornea inv	Presenting complaint	Other eye	Morphology-ical Type	Cytology correlation	Surgical procedure	Margins	HPE Diagnosis	Ki-67 index(%)	Node	Mets	Recur-rence
18	63/15	39	M	R	T	21	N	Y	growth	Normal	LEUK	C	Exentr	UI	SCC MD	41	N	N	N
19	78/15	55	F	L	T	6	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	11	N	N	N
20	82/15	80	M	R	N	17	N	Y	growth	Normal	PAP	C	Exc bx	I	SCC PD	40	N	N	N
21	114/15	55	F	R	N	5	N	Y	irritation	Normal	LEUK	C	Exc bx	UI	CIN III	18	N	N	N
22	144/15	67	M	R	T	13	Y	Y	growth	Normal	GEL	C	Exc bx	UI	SCC MD	38	N	N	N
23	151/15	75	M	R	N	4	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	24	N	N	N
24	239/15	68	M	R	N	2	N	Y	redness	Normal	GEL	C	Exc bx	UI	CIN II	20	N	N	N
25	368/15	65	F	R	N	12	N	Y	growth	Normal	PAP	C	Exc bx	UI	SCC MD	37	N	N	N
26	403/15	32	F	R	N	4	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	21	N	N	N
27	417/15	50	F	L	N	3	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	18	N	N	N
28	444/15	38	M	L	N	2	N	N	irritation	Normal	GEL	NC	Exc bx	UI	CIN I	13	N	N	N
29	567/15	40	M	L	T	6	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	17	N	N	N
30	572/15	68	M	R	N	7	N	Y	growth	Normal	LEUK	C	Exc bx	UI	CIN III	25	N	N	N
31	637/15	50	F	R	N	4	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	23	N	N	N
32	731/15	45	F	R	N	11	N	Y	growth	Normal	LEUK	C	Exc bx	UI	SCC PD	42	N	N	N
33	736/15	41	F	L	T	3	N	N	growth	Normal	GEL	C	Exc bx	UI	CIN II	16	N	N	N
34	77/16	66	M	L	T	3	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	19	N	N	N

S.No	HPE No	Age	Sex	Side	Site	Size mm	HIV +	Cornea inv	Presenting complaint	Other eye	Morphology-ical Type	Cytology correlation	Surgical procedure	Margins	HPE Diagnosis	Ki-67 index(%)	Node	Mets	Recur-rence
35	155/16	51	M	L	N	7	N	Y	growth	Normal	LEUK	C	Exc bx	UI	CIN III	22	N	N	Y
36	178/16	50	F	L	N	12	N	Y	growth	Normal	LEUK	C	Exc bx	UI	SCC WD	32	N	N	N
37	197/16	54	M	R	T	2	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	27	N	N	N
38	239/16	60	M	R	N	3	N	Y	redness	Normal	GEL	C	Exc bx	UI	CIN III	24	N	N	Y
39	240/16	40	M	L	T	3	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	20	N	N	N
40	266/16	63	F	R	N	17	N	Y	growth	Normal	GEL	C	Exc bx	UI	SCC MD	36	N	N	N
41	291/16	43	M	R	N	4	N	Y	growth	Normal	LEUK	C	Exc bx	UI	CIN III	21	N	N	N
42	430/16	45	M	R	T	3	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	20	N	N	N
43	437/16	46	M	R	N	5	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	27	N	N	N
44	538/16	42	M	R	N	2	N	N	growth	Normal	GEL	C	Exc bx	UI	CIN I	16	N	N	N
45	540/16	37	F	R	T	4	N	Y	growth	Normal	LEUK	C	Exc bx	UI	CIN III	22	N	N	N
46	646/16	70	M	L	T	22	N	Y	growth	Normal	LEUK	C	Exc bx	I	SCC MD	45	N	N	N
47	649/16	45	M	R	N	4	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN III	18	N	N	N
48	24/17	35	M	R	N	3	N	Y	growth	Normal	GEL	C	Exc bx	UI	CIN II	22	N	N	N
49	57/17	68	F	R	N	14	N	Y	growth	Normal	PAP	C	Exc bx	UI	SCC PD	39	N	N	N
50	63/17	75	M	R	T	2	N	Y	redness	Normal	GEL	NC	Exc bx	UI	CIN I	11	N	N	N